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WILLIAM KEITH BROOKS
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on the proliferous stolon. I remember with mortification how I floundered helplessly through the first few months in what appeared to me a hopeless struggle to reach solid ground, until one day I happened to find out something new about that stolon. It was a very trivial point, but in the exuberance of my first discovery I showed it to Professor Brooks, and from that moment his attitude toward me changed as if by magic. I was forthwith consecrated to the study of the Tunicata. Brooks had, however, the habit of suddenly suggesting and urging upon a student a totally different problem from the one upon which he was working, and this caused the greatest consternation among us during our earlier years, until we found by experience that he usually forgot about the matter in a few days. In this connection I cannot refrain from quoting from a letter which he wrote me from Baltimore while I was absorbed in studying the embryology of Appendicularia at the Beaufort Laboratory in the summer of 1895. "I have just heard from Bigelow," he wrote, "that the medusa which I have been studying (*Gonionemus*) is now abundant in the Eel Pond at Woods Hole. If you could get the embryology and metamorphosis, it would make a fine thesis, and I write in the hope that you may be disposed to go to Woods Hole at once to try to study it, and to get specimens of the adult for me." The idea of dropping all of my work and setting out on a journey from North Carolina to Massachusetts to collect jelly fishes did not appeal very strongly to me, and I remained in Beaufort, but just how I escaped from the situation, which was quite embarrassing at the time, I do not now remember.

The recollections of Professor Brooks that are the most vivid and interesting ones to me are chiefly associated with our summers at the marine laboratories, for it was there, away from the routine and greater restraint of the life in Baltimore, that we came to know him most intimately and affectionately. In the daily companionship with him, for he constantly shared with us both the joys and hardships of the work, the lovable side of his nature was conspicuously open to us. A thousand incidents associated with him at Beaufort crowd my memory as I recall him there, the center of our life, the enthusiastic naturalist, the wise coun-

seller and teacher, the sympathetic friend, his droll humor always in evidence, but with never a trace of unkindness. I remember a day when one of the men, a rather puritanical student, who had been struggling with some refractory material, in a moment of discouragement told Professor Brooks that he could do nothing with it. In his characteristic way he made no reply at the time, but some hours later returned and said quietly, "Did you ever try swearing? That helps sometimes."

Most delightful of all is the recollection of long evenings on the verandah, where, after the day's work was done, we sometimes sat listening to his talk on nature and philosophy. True it is that we were not always able to follow him closely in his metaphysical moods but we learned at least to feel something of the relation that exists between the study of phenomena and the philosophic inquiry into underlying causes.

1900-05.¹ To work on aphids, to read Witlaczil, these were my first instructions. After that he seemed to have lost interest in me, and he showed none in aphids. Months later he startled me by suddenly proposing three elaborate dissections, a study of the lamellibranch gill, and of the brooding habits of *Cyclas*.

To improve his pedagogy seemed an easy thing at that time; to-day I am thankful that he left me alone, and neither pushed nor pulled. Into the sea of work suggested I plunged, but Brooks furnished no life-belts. Instead he gave opportunity, and something more.

In my time the "Foundations" were being read, discussed, and not wholly understood. The typewriter in the laboratory and at Brightside, clicked incessantly. The Lowell Lectures were in the making; bulky translations from Hertwig and from Heider were completed though never published; many essays and shorter papers were written; Berkeley was quoted; and the pile of incoming reprints remained unclassified on the floor.

He seemed to be writing much, and the larger problems, for the time, triumphed over the microscope. The doom of his morphological studies was practically sealed by illness that grad-

¹ Professor Otto C. Glaser, University of Michigan.

ually became worse, until his system, enfeebled by a weak heart, scarcely resisted the other difficulties that began to burden him. It is true that periods of improvement alternated with those of depression, but the doubt that hovered over him cast its shadow through the laboratory. It was at this time that Mrs. Brooks died.

Such health as he had known, never came back wholly, and months passed before interest in life and work returned. With renewed vigor he studied his hydroids, his salpas, and the oyster, began to complete researches half-forgotten, and to start new ones. The Sunday evenings at Brightside, too, were resumed, and amid clouds of smoke, he read Berkeley or his own writings. This was Indian summer.

At home, much of his most vital teaching was done. Stretched comfortably in his steamer chair, in full view of the books and pictures that he loved, and surrounded by a family, not in the narrow sense, but one in which his students, his negro servants, his dogs, and his flowers had each a place, he was thoroughly at ease. Often, as he laid his hand affectionately on Jupe's great head, he spoke with tenderness of the details of his home-life.

If one thing must be singled out to explain the affection he inspired, it is that he himself was affectionate. The loyalty that led him to give of his own small income in times of need and made him speak of former students as though they had been with him only yesterday, included other things, his science, his duties as a teacher, and his university. In its period of hardship he economized, and offers from other institutions did not shake him.

His interests were human, and his science a pathway along which he walked in humility to view the world and to interpret it. The great problems were not mere exercises for the mind, but human difficulties. The teacher and the man were inseparable and it was no less the man than the teacher who inspired others.

1905-08.⁸ It was during Professor Brooks' declining years that he honored me with his friendship. On these visits of his to the

⁸ Dr. A. G. Mayer, Carnegie Institution of Washington.

Carnegie Laboratory at Tortugas I was much impressed with his broad kindness and tolerance of spirit and with his interest in the world. The force and independence of his character also were obvious and it was clear that he would have been a deep student of living things under any conditions of life. He was a thinker even more than an observer. He was the follower of no school, and few men have been so little dominated by the thoughts of the world around them.

Still it was not his power and originality alone that made him great and revered among us. It was his spirit that led us onward in our science. The little boy who studied dragon flies in the pool of his father's yard had had many years pass over him, yet in his simple wondering love of nature he remained as in his childhood days. This deep reverence for the universe of which he felt he formed so small a part, made him careless of many things we deem important in our daily life, for his thoughts were not upon things of the moment but were far beyond in the borderland between the known and the unknown.

THE CHESAPEAKE ZOÖLOGICAL LABORATORY^{*}

Professor Brooks' early experience at Penikese under Louis Agassiz must have had a great effect upon him. From that time on his interest in marine zoölogy was one of the dominant influences in his life. One of his first important acts at the Johns Hopkins University was to organize (in 1878) a movable seaside station under the name of the Chesapeake Zoölogical Laboratory and during the following twenty-eight years he was constantly to be found during the warmer season at some point on the coast or in the West Indies accompanied by a party of students, all engaged in the study of marine life.

The importance of this Laboratory in the development of the biological department of the Johns Hopkins University and in the general advance of zoölogy in America may be estimated from the large number of students who worked at the laboratory and

^{*} Professor E. G. Conklin, Princeton University, in *National Academy Biographical Memoirs*, vol. 7.

the large number of papers which they published. Doctor Brooks expected all of his graduate students to spend a season or more at this laboratory. He rightly estimated such work as the most valuable experience a beginner could have, for in this way the student became acquainted with animals under natural conditions; he had the opportunity of laying a broad foundation for his future work as a naturalist, of finding for himself some matters to investigate, and thus early to acquire the mental habit of the independent investigator.

The Chesapeake Laboratory, as said, was not limited to one place. For the first few years of its existence it was located at several different points on Chesapeake Bay; afterwards it was located at Beaufort, North Carolina; then at different places in the Bahama Islands, and finally in Jamaica. In the various expeditions of Brooks and his students to these different places they made not only a biological survey of each region, but they did work of most fundamental and far-reaching importance on the various groups of animals found. Out of these expeditions has grown the beautiful and permanent station of the U. S. Fisheries Bureau at Beaufort, North Carolina, in which Brooks took great interest and pride. It was on these expeditions that his students came to know him most intimately and affectionately. In the memory of each of them is fixed some scene of his enthusiasm over the discovery of a rare form or of an unknown stage in some life history; his long vigils full of exciting discoveries; his quiet talks on nature and philosophy.

The Chesapeake Zoölogical Laboratory occupied so large a place in the life and work of Professor Brooks that it seems desirable to reproduce here, in his own words, a more detailed account of the aims and history of that laboratory during its first nine years. The following is taken from a report by Professor Brooks on "The Zoölogical Work of the Johns Hopkins University, 1878-86," published in the Johns Hopkins University Circulars, vol. 6, No. 54:

In natural science the policy of the University is to promote the study of life, rather than to accumulate specimens: and since natural laws are best studied in their simplest manifestations, much attention has been

given to the investigation of the simpler forms of life, with confidence that this will ultimately contribute to a clearer insight into all vital phenomena.

The oldest forms of life are marine: every great group of animals is represented in the ocean, while many important and instructive groups have no terrestrial representatives; omitting the insects, more than four-fifths of the known species of animals are marine, and the total amount of animal life in the ocean is incomparably greater than upon the land. In a word, the ocean is now, as it has been at all stages in the earth's history, the home of life; and it is there, and there only, that we find the living representatives of the oldest fossils, and are thus enabled to study the continuous history of life from its simplest to its most complex manifestations.

On the sand flats at the mouth of the Chesapeake Bay, we find, living side by side, animals like *Lingula*, *Amphioxus*, *Limulus* and *Balanoglossus*, which are the representatives of some of the oldest and most primitive types of animal life; and all attempts to trace out the natural relationships of any group of animals, lead us at once to forms which are found only in the ocean.

The animals which have contributed most extensively to the formation of the earth's crust, the corals and foraminifera and radiolarians, abound in the ocean to-day, and it is only by studying their life, by observations at the seashore, that we can understand and interpret their geological influence.

Nearly every one of the great generalizations of morphology is based upon the study of marine animals, and most of the problems which are now awaiting a solution must be answered in the same way.

For these reasons our chief aim in zoölogy and animal morphology has been to provide means for research upon the marine animals of the Atlantic coast, and for nine years, successive parties, composed of instructors, fellows and students in this department, together with instructors and advanced students from other institutions have spent at the seashore all the months in which marine work is practicable. Their time and energy have been devoted to research rather than to the preservation of collections, and the wisdom of this course can be estimated by examination of the accompanying list of publications [here omitted]; all of which are based, either in part or entirely, upon researches which we have carried on at the seashore.

The wisdom of our policy is well illustrated by the fact that the leading naturalist of America, himself the head of one of the largest scientific

collections in the world, says in his annual report for 1884,¹⁰ that the expenses of an immense natural-history collection are so great that it would be far cheaper, with the present facilities and the cost of travel, to supply the student with the necessary funds for valuable researches, than to go on for years spending in salaries of curators and the care of collections, sums of money which, if spent in a different manner, in promoting original investigation in the field or in the laboratory and in providing means for the publication of such original researches, would do far more towards the promotion of natural history than our past methods of spending our resources.

This fact has become widely recognized during the last ten years, as is shown by the establishment of marine laboratories by several of the European institutions of learning; and in the summers of 1883 and 1884 we had with us at our laboratory a young English naturalist (Wm. Bateson) who had been provided by the Royal Society of London with funds for his researches, the results of which have recently been published in England.

The Johns Hopkins University was among the first to recognize and act upon this new departure in zoölogy, and our little marine station is almost as old as the great Naples laboratory. Briefly stated its history is as follows:

In 1878 a small appropriation was made to enable a party of biologists from the University to spend a few weeks at the seashore in the study of marine zoölogy. Through the influence of Maj. Gen. Q. A. Gillmore, the Secretary of War permitted us to occupy the vacant building at Fort Wool. Prof. Spencer F. Baird also exerted his influence with the Secretary of War in our behalf, and aided us in many other ways; furnishing us with dredging apparatus and with three small row-boats. The scientific results of our season's work were printed in an illustrated volume, the cost of publishing which was borne by the following citizens of Baltimore: Samuel M. Shoemaker, John W. Garrett, John W. McCoy, Enoch Pratt, P. R. Uhler, T. B. Ferguson, Dr. Geo. Reuling, President Gilman, Professor Martin and others.

In 1879 the appropriation for the maintenance of the laboratory was renewed, and in order to present an opportunity for studying the oyster beds of Maryland, the laboratory was opened in three of the barges of the Maryland Fish Commission at Crisfield, Maryland, a point which proved to be very unfavorable. Maj. T. B. Ferguson, the State Fish Commissioner, not only provided the barges for our accommodation,

¹⁰ Report of the Museum of Comparative Zoölogy, Cambridge, Mass.

but he also fitted the steam yacht *Lookout* with dredging apparatus, and rendered us valuable help in dredging and collecting. Through his influence a small steam launch was also detailed from the U. S. Navy for our use.

The next year the Trustees of the University voted to continue the laboratory for three years more, 1880-1-2, and they provided a liberal annual appropriation of \$1,000 for current expenses, which was renewed annually in 1883-4-5-6, and was expended in rent, wages, fuel, laboratory supplies, repairs, etc. They also appropriated the sum of \$4,500 for permanent outfit, and most of this was used in the purchase of two boats; a Herreshoff steam launch twenty-seven feet long and eight feet beam, and a center-board sloop forty-seven feet long and fourteen feet beam.

After an examination of all the available localities the town of Beaufort, N. C., about four hundred miles south of Baltimore, was selected as the site for the laboratory, and a vacant house, suitable for the accommodation of a small party, was found and rented as a laboratory and lodgings for the party, and it has been occupied during the seasons of 1880-1-2-4-5, and by two students in 1886. As the director was, in 1883, a member of the Maryland Oyster Commission, the outfit of the laboratory was that year moved from Beaufort into the Chesapeake Bay, and we occupied a building which we rented from the Normal School at Hampton, Va. As Hampton proved to be a very unfavorable place for our work we returned to Beaufort the next year, and we have accordingly spent five seasons at Beaufort.

During the season of 1886 the zoölogical students of the University were stationed at three widely separated points of the seacoast. A party of seven under my direction visited the Bahama Islands, two were at Beaufort, and one occupied the University table at the station of the U. S. Fish Commission at Woods Hole.

The party which visited the Bahamas consisted of seven persons, and our expedition occupied two months, about half of this being consumed by the journey.

The season which is most suitable for our work ends in July, and we had hoped to reach the Islands in time for ten or twelve weeks of work there, but the difficulty which I experienced in my attempts to obtain a proper vessel delayed us in Baltimore, and as we met with many delays after we started, we were nearly three weeks in reaching our destination.

We stopped at Beaufort to ship our laboratory outfit and furniture, but the vessel, a schooner of 49 tons, was so small that all the available space was needed for our accommodation, and we were forced to leave part of our outfit behind at Beaufort.

We reached our destination, Green Turtle Key, on June 2nd, and remained there until July 1st. The fauna proved to be so rich and varied and so easily accessible that we were able to do good work, notwithstanding the shortness of our stay and the very primitive character of our laboratory. This was a small dwelling house which we rented. It was not very well adapted for our purposes, and we occupied as lodgings the rooms which we used as work-rooms.

Record of the various sessions

For the following brief records of the various sessions we are indebted in large part to Prof. E. A. Andrews.

- 1878: 8 weeks, Ft. Wool, Virginia; 7 members. Brooks studied embryology of Lingula.
- 1879: June 25–August 8, Crisfield, Maryland; 11 members. Brooks studied the oyster. Three barges served as laboratory and quarters. Swarms of mosquitos led to the abandonment of this locality early in August, and the removal of the laboratory to Ft. Wool, until September 15.
- 1880: April 23–September 30, Beaufort, North Carolina; 6 members. Laboratory and quarters were in the Gibbs house. A steam launch was bought and the laboratory equipped by means of an appropriation from the University.
- 1881: May 2–end of August, Beaufort, North Carolina; 12 members. An "Elementary Seaside School" had been announced, with lectures by Brooks and S. F. Clarke; fee for the course, \$25.
- 1882: May 1–end of September, Beaufort, North Carolina; 8 members.
- 1883: May 1–October 1, Hampton, Virginia. As a member of the Maryland Oyster Commission Brooks was obliged to spend this summer on the Chesapeake. The new machine shop of the Hampton Institute was rented as a laboratory, and a fast sloop was added to the equipment. Wm. Bateson there joined the party to study the development of Balanoglossus.
- 1884: June 1–September 19, Beaufort, North Carolina; 10 members. The illness of Brooks obliged him to return after a month, leaving the laboratory in charge of H. W. Conn. Bateson, who was again with the party, was also seriously ill.
- 1885: May 23–September 15, Beaufort, North Carolina; 11 members. Brooks became a licensed pilot to take the steam launch in and out of Beaufort Inlet.

- 1886: June 2–July 1, Green Turtle Key, Abaco, Bahamas; 7 members. The party left Baltimore, May 1, in a small Bay schooner, chartered by the day, with Brooks as pilot. With head winds, mishaps and a stop at Beaufort to take on laboratory furniture they did not reach their destination until June 2.
- 1887: March 1–July 1, Nassau, Bahamas; 12 members. After this session, owing to financial losses on the part of the University, the Chesapeake Zoölogical Laboratory was temporarily suspended and its outfit dispersed.
- 1888 and 1889: Brooks, with some of his students, was at Woods Hole, Massachusetts, as naturalist in charge of the U. S. Fish Commission Station.
- 1891: May 26–September 1, Kingston, Jamaica; 15 members. The Chesapeake Zoölogical Laboratory was established at Port Henderson, on the harbor opposite Kingston.
- 1892: A party of three, in charge of Professor Andrews, was located at Alice Town, North Bimini, Bahamas. Brooks did not go.
- 1893: April 20–July 23, Port Henderson, Jamaica; 7 members. Brooks did not go and Dr. R. P. Bigelow was acting director.
- 1894: April 7–July 7, Beaufort, North Carolina; 9 members. Brooks was present.
- 1895: June 6–August 13, Beaufort, North Carolina; 4 members. Doctor Sigerfoos was acting director; Brooks was not present.
- 1896: April 29–July 30, Port Henderson, Jamaica; 4 members. Dr. F. S. Conant was acting director; Brooks was there for a while.
- 1897: June–September, Port Antonio, Jamaica; 12 members. Prof. James Ellis Humphrey was acting director. Humphrey died there of yellow fever, August 12; Dr. Franklin Story Conant contracted the fever there, and died on his return to Boston in September.
- 1898: Beaufort, North Carolina; 6 members. Prof. H. V. Wilson was director. In this and all subsequent years students went, with little or no aid from the University, to the U. S. Fish Commission Station at Beaufort.
- 1901–1906: Brooks was again at Beaufort in 1901 and 1903, and at the Marine Laboratory of the Carnegie Institution at Dry Tortugas, Florida, in 1905 and 1906.

PROFESSOR BROOKS AS AN INVESTIGATOR AND WRITER

Professor Brooks' investigations lay mainly in the field of animal morphology and embryology. In this field he was an acute observer possessed of great patience and pertinacity. His philosophic insight and breadth of view, moreover, made him alert to the significance of what he observed, and his memoirs are hence notable for their suggestive and broad theoretical discussions. Fundamental resemblances in the development and anatomy of forms were the phenomena in which he was especially interested. Interrelationships between groups and the phylogenetic value of embryonic and larval characteristics were the speculative problems on which he brought his discoveries to bear. In reaching conclusions from facts he showed the caution of the observer who had seen much, and his soundness of judgment is widely recognized. Nevertheless he was at times not averse to bold speculation, as may be seen in his instructive discussion of the nature of the early pre-cambrian fauna, and the origin of the existing great groups of animals (The Genus *Salpa* and The Foundations of Zoölogy). His morphological studies embraced a number of invertebrate groups, pelagic tunicates, mollusks, molluscoidea, crustacea, and hydromedusæ.

The illustrations in Brooks' memoirs are striking. It was his practice to make them himself, and they have the artistic excellence combined with truthfulness of detail found only in the work of the artist-naturalist. Most of his drawings were in pen and ink, the shaded parts stippled, and made on a large scale suitable for reduction. They represented much labor, but Brooks was a quick worker in this style, which he preferred above all others. The mechanical process of stippling aided him, he maintained, to abstract his mind and to follow out lines of thought quite unrelated to the drawing. With respect to his artistic skill Brooks was without egotism, and when the drawings were once reproduced the originals were thrown away.

Together with skill in drawing Professor Brooks was unusually fortunate in possessing literary power in a marked degree. His subject is presented in an order and manner that makes it easy

for the reader to follow, and his command of language is admirably suited to the needs of the naturalist. His technical papers always show order and proportion and a fine precision in the use of words. These qualities appear too in his one text-book, the "Handbook of Invertebrate Zoölogy," a manual so excellent that it has been a model for many later books in this field. His popular articles and lectures reveal the same logical habit of mind, but in these it is the graphic description that especially seizes the mind of the reader. Particularly pleasing and effective are the descriptions of scenes of nature in which animals dominate, or of the behavior of individual animals. In the argumentative portions of his later writings dealing with heredity and the philosophical aspects of nature, Brooks is not always easy to follow. He leaves a good deal of responsibility on the reader. Yet these writings contain much that is beautiful in style as well as in idea, much that is very quotable, real "nuggets of wisdom, products of deep thought as well as of careful observation."¹¹

*Researches on the Tunicata.*¹² Brooks' first contributions on the Salpidæ appeared in 1875-'76. He observed that the eggs, which are borne by the individuals of the chain, arise really in the solitary Salpa and are passed into the stolon early in its development. Each individual of the chain receives usually one (in some species more) of these eggs and serves as nurse to the embryo which comes from it. Salpa, therefore, does not show true alternation of generations, and Charnisso's discovery of an apparent metagenesis in this form must be looked on as a misinterpretation of the phenomena. In his later study Brooks found that the spermatozoa as well as the eggs come from the mass of germ cells lying in the ventral part of the solitary Salpa, so that the solitary Salpa is in reality a potential bisexual animal.

Brooks worked out in far greater detail and with greater clearness than any other student the development of the buds upon the stolon, and showed the fundamental harmony of the process of

¹¹ President D. S. Jordan.

¹² Professor M. M. Metcalf, Oberlin College.

budding in *Salpa* with that in *Pyrosoma* and in the *Clavelinidæ* among the ascidians. The stolon is bilaterally symmetrical, its planes of symmetry coinciding with those of the solitary *Salpa* which bears it, and at first the planes of symmetry of every member of the chain coincide with those of the stolon and the solitary *Salpa*. Very soon, however, a twisting of the chain occurs which leads to the formation of a double row of *Salpæ*, each row with the dorsal surfaces of its members turned outward while the ventral surfaces of the two rows are turned toward one another, and the right sides of the members of one row and the left sides of those of the other row are turned toward the base of the stolon.

He showed that the placenta of *Salpa* does not resemble the mammalian placenta in its method of nourishing the embryo, but that certain cells in the placenta, taking nourishment from the blood stream of the nurse (the chain *Salpa*), grow to very large size, then lose their connection with the placenta and wander to different parts of the embryo, where they break down and nourish the growing tissues of the embryo.

Salensky's generally accurate work upon the embryology of many species of *Salpa* contained one fundamental error, since he described the embryos as arising not from true blastomeres but from follicle cells, the blastomeres degenerating early in the developmental history. Brooks, recognizing the improbability of any such conditions, succeeded in tracing the development of the egg itself until from its blastomeres the organs arise. He found that the blastomeres develop very slowly; that the follicle cells, on the other hand, proliferate very rapidly and take on the form of the rudiments of the several organs, the organs being thus blocked out in these extra-embryonic cells, while as yet the blastomeres are very few in number. Later the blastomeres multiply and pass into the different parts of the mold thus formed for them by the follicle cells, and gradually use as food the degenerating follicle cells that surround them.

In his latest, unpublished work he traced the cleavage of the egg; he found a clear gastrula arising by invagination from the group of blastomeres; he observed the hollow dorsal nerve tube, finding it at first considerably elongated; he found a postero-dorsal rod

of blastomeres, the notochord, which later for the most part passed into a postero-ventral protuberance there to degenerate and share in the formation of the eleoblast which he thus clearly showed to be a degenerate tail; at one time he was studying structures in some of his embryos which seemed to be a pair of true stigmata, but his final decision in regard to them is unknown.

Brooks' embryological work convinced him that Salpa, though now perfectly adapted for pelagic life, has not always been pelagic, but that it is descended from sessile forms like the ascidians, and that some of the features which so well adapt Salpa for a pelagic existence arose during this sessile stage in its ancestry, or were then much improved over the earlier condition illustrated in Appendicularia. Having found this most typically pelagic of all pelagic animals to be a migrant from the ocean bottom, he was led to review the whole pelagic fauna, and as a result of this review reached the conclusion that nearly all pelagic animals of considerable size or complex structure have had a similar history and are descended from forms that once lived on or near the ocean bottom.

The memoirs upon the Salpidæ are of such comprehensive character and fundamental importance that they must be designated as monumental. This massive character of his work, together with the soundness of judgment displayed, has unquestionably made Brooks the foremost student of the group. It is he, more than all others, who succeeded in showing that beneath the perplexing maze of secondary phenomena which so obscures the development of this group, there is a general conformity to the development of other chordates.

*Researches on the Crustacea.*¹³ Professor Brooks' interest in the Crustacea began early, for as a boy he had collected the freshwater shrimp, *Palaemonetes exilipes*, in the Rocky River near his Cleveland home, and in the marine laboratory of Alexander Agassiz he had observed with astonishment "the lively interest in shells," displayed by the newly hatched hermit crabs. That

¹³ Professor F. H. Herrick, Western Reserve University.

the impressions thus made were strong may be gathered from the fact that many years later he urged the writer to study the development of this shrimp, if possible, and that after the lapse of nearly a quarter of a century he wrote the graphic account of the behavior of the hermit crab which appears in the introductory lecture of his work on "The Foundations of Zoölogy."

Altogether there are about fifteen papers on the embryology, metamorphosis, habits and classification of the higher crustacea which singly or jointly bear the name of Brooks, and all were issued during a period of fourteen years, from 1879 to 1892. Moreover, his "Handbook of Invertebrate Zoölogy" contains much original matter pertaining to this class of animals. His first contribution in this field was on the larval stages of the stomatopod, *Squilla empusa*, and represented the first "Scientific Results of the Chesapeake Zoölogical Laboratory" for 1878, and it was upon the adults and the larvæ of this sub-order that some of his most notable work was later accomplished.

At Beaufort, North Carolina, during the season of 1880, Brooks' interest in Crustacea deepened, for he saw in their structure and in the metamorphosis which they so beautifully displayed, a means of attacking several larger problems, such as "the laws of larval development," the analysis of secondary adaptations, and the meaning of metamerism in both the lower and higher animals. He had pondered over the works of Professor Claus on crustacean development and morphology, and for upwards of four years, from 1880 to 1883, his own elaborate notes and pen drawings on the *Macrura* had grown to such an extent that they filled a large portfolio. From this source he drew materials from time to time for publication, as certain subjects happened to engage his special attention. Without doubt he had contemplated an extended monograph, which was only partially fulfilled in the work on "The Embryology and Metamorphosis of the *Macrura*," published in 1892.

The works by which Brooks will be best known to all future students of crustacean zoölogy are undoubtedly his monograph on "*Lucifer: A Study in Morphology*," published in the *Philosophical Transactions of the Royal Society of Great Britain* for

1882, and his "Report on the Stomatopoda," which appeared as part of the sixteenth volume of the Scientific results of the Challenger Expedition in 1886. In the former work, we are told that in April 1880, he found at Beaufort "a single Lucifer with two eggs attached to one of its appendages," and that he was "led by the great importance and interest of the subject to make every effort to trace its life-history." Success came only after months of repeated failure, when at last he could say with evident satisfaction: "I have seen the eggs of Lucifer pass out of the oviduct. I have seen the Nauplius embryo escape from the same egg which I had seen laid, and I have traced every moult from the Nauplius to the adult in isolated specimens. There is therefore no crustacean with the metamorphosis of which we are more thoroughly acquainted than we now are with that of this extremely interesting genus." Not only did he discover that Lucifer emerged from the egg as a true Nauplius, but what was even more novel, that the egg underwent a total and regular segmentation, and gave rise to an egg-gastrula of the invaginate type. After giving an exhaustive analysis of the developmental stages of Lucifer, and comparing its successive appendages with those of other representative Malacostraca, he concludes that the three-jointed Nauplius larva represents a true ancestor, that there is essentially but one kind of homology presented by metameric animals, and that, therefore, the remote ancestor of the crustacea does not represent a community of once independent parts.

The monograph on the Stomatopoda is distinguished by the great ingenuity shown in classifying all of the known larvæ of this sub-order, and in tracing them to their proper genera, for he had no living material to work with, excepting the two species from the southern coast of the United States, *Squilla empusa* and *Lysiosquilla excavatrix*, which he had previously studied, and which he used for exact comparisons so far as possible. He said of the collection submitted to him, that while it contained only fifteen species of adults, eight of which were new, it was very rich in larvæ. In speaking of the eggs, he remarked that since they were not carried about by the female, attached to her body or appendages, as is the rule in the higher crustacea, they quickly

perished when deprived of the constant current thus supplied, and that it was very difficult to procure them at all, adding that he knew of no young Stomatopod which had been reared from an egg outside the burrow or in an aquarium.

The sentence just quoted was written in 1885, and we can appreciate the pleasure he must have experienced in being able to do the very thing to which he alludes, two years later at Nassau, for on the first or second day after reaching the Bahamas one of his students, Dr. E. A. Andrews, brought him "a *Gonodactylus* and a bunch of yellow eggs," which had been broken out of a coral rock. Feeling sure that at last he was on the track of a stomatopod's eggs, he started at once for the beach, and it was not long, as he tells us, before "the problem was solved, and I went home and to bed, confident that I should next day get all the embryological material I needed." The notable paper in which he has described how a stomatopod crustacean was for the first time reared from an egg, and followed in all its successive stages, alive, should not be overlooked, though appearing as a chapter in another work (*The Embryology and Metamorphosis of the Macrura*. Chapter III.).

Researches upon the Cœlenterata.¹⁴ Exclusive of preliminary accounts afterwards published in more amplified form, and of popular writings, Professor Brooks produced either alone, or in coöperation with his students, ten papers upon cœlenterates. All are the results of labors of his maturity, for he was thirty-two years of age when the first was published. This may account in some measure for the high standard he maintained throughout these papers, for next to Agassiz we must rank him as the greatest student of the cœlenterates of our country.

The excellence of his work depends not upon the number of species he described as new to science, for of these he names but eleven during the whole twenty-seven years covered by his writings on cœlenterates. It is in the fields of embryology and anatomy that Brooks' work stands preëminent; and his life-histories of

¹⁴ Dr. A. G. Mayer, Carnegie Institution.

Liriope, Cunoctantha, Eutima, and Philalidium McCradyi are classics of science in their thoroughness, wealth of accurate illustration, and that subtle charm in description which was their author's own. Through patient searching upon many a collecting trip at Beaufort, he was the first to find and describe the hydroids of Turrítopsis, Nemopsis, Phortis, and Stomotoca, while his studies along the shores of the Chesapeake led to discovery of the ephyrae and early growth-stages of the free swimming medusa of Dactylometra.

His summers in the Bahamas led to the discovery of the remarkable process of the development of medusa-bearing hydroid blastostyles upon the gonads in Epenthesia (Philalidium) McCradyi. He also sectioned and beautifully figured, the marginal cordyli of Laodicea, and was the first to elucidate their structure and homologies; and from the standpoint of morphology his description of Dichotomia cannoides in the Proceedings of the American Philosophical Society of Philadelphia, 1903, may well serve as a model for those who essay to describe medusæ.

It was in coöperation with Brooks that Conklin discovered that in Physalia only male gonophores are found, while in another siphonophore, Rodalia, only female gonophores occur, the inference being that in both forms the opposite sex is so different from the one known that it may have been classed as a wholly different genus. Another of his students, Rittenhouse, while working under Brooks, gave an excellent account of the early stages of the development of Turrítopsis.

Facts interested him but little unless they led toward generalizations, and thus it is that he wrote but one purely systematic paper upon cœlenterates, and that all of his other work was directed toward the study of developments and homologies as indicating what has been the path of evolution. Such a problem as the relationship between cœlenterates and bilateral animals was accordingly very attractive to him, and he was disposed to lay stress upon the (somewhat masked) bilateral symmetry discovered by himself in Eutima and by Hamann in other hydroids.

Brooks' views were not seldom in conflict with accepted theories, as when in 1886 he came to the conclusion that the remote ances-

tor of the hydromedusæ was a solitary free-swimming hydra or actinula with no medusa-stage but probably with the power to multiply by budding. Finally, however, becoming more perfectly adapted to a swimming life it was converted into a medusa with pulsating bell, and with sense-organs. After this the larva derived an advantage through attachment, and thus the hydroid stage was secondarily produced, and then perpetuated through natural selection. It may be said of this theory that while it has gained no important following, yet nevertheless it has never been disproven. It is logically sound, presents the direct development of certain medusæ from a new point of view, and the future may possibly show that it rests on a basis of truth.

*Researches on the Mollusca and the Molluscoidea.*¹⁵ Two of Brooks' first papers deal with the lamellibranchs, one (1874) with an "organ of special sense" in *Yoldia*, while in the other (1875) the development of *Anodonta imbecilis* is described in outline, and the conclusion is reached that the larva, *Glochidium*, is a specially modified stage and has no bearing on the question of the origin of the group. In a paper "On the Affinities of the Mollusca and Molluscoidea" (1876) he again approached phylogenetic problems, and concluded that the Brachiopoda have been derived from Vermes, Polyzoa from Brachiopoda, and the molluscan veliger (prototype of the Mollusca) from Polyzoa. Later in his paper on the development of *Lingula* (1879) he held that the Rotifera, Polyzoa, and Veliger were three branches which early diverged from the vermian stem. The Brachiopoda he held to be the most highly specialized members of the polyzoan branch, the Mollusca the most highly specialized of the Veliger branch. For these three branches he proposed the name Trochifera.

In his "Observations on the Early Stages in the Development of Fresh-Water Pulmonates" (1879) he observed the rhythmical nature of the process of cleavage, and devoted considerable attention to the origin of the germ layers, to the fate of the blasto-

¹⁵ Professor G. A. Drew, University of Maine.

pore, and the origin of the digestive tract. The technique necessary for the successful sectioning of such small bodies as snail eggs had not been developed at this time. Brooks' observations were therefore made exclusively on material studied in toto, and it is interesting to find that this method of study led him into several serious errors. In his paper on the "Acquisition and Loss of a Food Yolk in Molluscan Eggs," Brooks devoted much attention to what is now known as the yolk lobe, or polar lobe, which he regarded as a food yolk which is disappearing in some forms, while in others it is being acquired. In a brief paper on the "Development of the Digestive Tract in Mollusks" he reiterates his mistaken view that in gasteropods and lamellibranchs the blastopore is converted into the shell gland. Not until 1908 did he return to the gasteropods, publishing in that year in association with Bartgis McGlone, one of his students, a paper on the origin of the lung in Ampullaria.

Brooks has two papers on the development of cephalopods published in 1880. His important conclusions in these papers deal with the homologies of the cephalopod yolk sac, siphon, and arms. Numerous publications deal with the development and propagation of the oyster. In 1878, during the first session of the Chesapeake Zoölogical Laboratory, he attempted to find young oysters in the gills of the female, as had been described for the European oyster, but without success. In May, 1879, he went to Crisfield, Maryland, the center of the oyster industry on the Chesapeake, and settled down to study the problem of the development of the oyster. He soon learned that artificial fertilization was possible, and that the American oyster normally discharges its eggs and sperm into the open water, where the processes of fertilization and development go on independently of the parents. The results of his embryological studies on the oyster were published in full in a report to the Maryland Fish Commission (1880). This paper was very favorably received and was republished in whole or in part in many American and European journals. In recognition of the importance of this work he was awarded a medal by the Société d'Acclimatation of Paris.

*Economic Work on the Oyster Fisheries.*¹⁸ Brooks' economic work on the oyster began in 1882 when the Governor of Maryland appointed him Chairman of a Commission to examine the oyster beds and to advise as to their protection and improvement. While occupying this position, he was excused by the Johns Hopkins University from practically all duties as teacher and investigator and for two years he devoted his talents and energy to the work of the Commission. He organized and carried on an extensive investigation of the actual condition of the natural oyster beds of Maryland and studied carefully the results of the policy then pursued by the State in its work of supervising and policing its oyster resources. He also compiled statistics from the history of the oyster industries of France and the North Atlantic States in order to ascertain and to show the possibilities of oyster production possessed by the tide waters of Maryland and the conditions under which some of these possibilities may be realized.

A detailed account of these investigations was published in January 1884 under the title "Report of the Oyster Commission of the State of Maryland" (a quarto volume of 193 pages), and carefully prepared plans for inaugurating a system of oyster culture under private ownership and for increasing the supply of oysters from the public oyster grounds, were submitted to the General Assembly for its consideration and approval.

The plans worked out by Brooks by which the oyster resources of the Chesapeake Bay and its tributaries could be husbanded and developed, were far in advance of public sentiment in Maryland and were rejected. Not until 1906, twenty-two years later, did the Legislature enact a general oyster culture law for the entire State.

Professor Brooks' active interest in the Maryland oyster problem did not end when his connection with the State Commission expired. He realized from the character of the discussion and opposition which brought about the rejection of his plan for oyster culture, that the oyster problem in Maryland is in reality a social and political one, and he therefore set about conducting a

¹⁸ Professor Caswell Grave, Johns Hopkins University.

long campaign of education. In this he had in mind to bring the State to realize the value of the Chesapeake for oyster production, and the worth of proper methods of supervision and cultivation. He was available for semi-popular lectures, wrote magazine and newspaper articles on the subject of oysters, and in 1891, published a treatise entitled "The Oyster," a little book that had wide influence and which was characterized by President D. C. Gilman as "a memoir in natural history and a chapter of political economy," in which the life history of the oyster is described "in terms scientific enough to be accurate, not so scientific as to be hard of understanding."

Dr. Brooks' efforts during this period resulted in the creation of an intelligent appreciation on the part of the general public not only throughout Maryland but in all of the Atlantic States as well, of the value and possibilities of the natural resources of tidal waters for the production of oysters, and men of large influence were enlisted in the cause of oyster culture. This deep cumulative influence of Professor Brooks on the public mind made him one of the most valuable citizens Maryland has ever had. Others carried to completion the task of crystallizing sentiment in favor of oyster culture, and finally in 1906 the Maryland Legislature passed a law for the protection and propagation of oysters along substantially the line that had been advocated by Brooks. The long campaign was thus happily terminated.

*Contribution to Anthropology.*¹⁷ Brooks' paper "On the Lucayan Indians" embodies the results of an excursion into the field of physical anthropology made during two visits to the Bahama Islands in connection with his summer laboratory. Very characteristically, he became interested in the history of the islands and in the people who dwelt there when they were discovered by Columbus. The skeletal fragments which there is reason to believe represent remains of the aborigines are very few. The material which Brooks had, and which was found in caves on the islands, consisted of three well preserved skulls and some

¹⁷ Professor H. H. Donaldson, The Wistar Institute.

other bones and fragments of bones. From these he was able to determine several of the more important physical characters of that ill-fated people who have left but a single monument, the word "hammock."

In the paper in question, which was read before the National Academy in November, 1887, Brooks gives a series of admirable plates artistically illustrating the skulls described, and reaches several conclusions, which may be summarized as follows: The bones are thick, massive and dense; the skulls are of good size. They are highly brachycephalic but at the same time artificially deformed in a way which would increase their brachycephalic shape. There is no reason to think that the people were gigantic, though they were probably of large size. From certain similarities to the remains of the inhabitants of southern Florida, it is probable that the Lucayans belonged to the same race.

*Studies on Heredity.*¹⁸ As early as 1876 in a paper entitled "A Provisional Hypothesis of Pangenesis" Brooks began to deal with questions of heredity and variation. His thinking in this direction took shape and led in 1883 to the publication of a volume under the title of "The Law of Heredity." The central point in the theory here presented is the conviction that the reproductive elements are, contrary to the usual opinion, not alike in function. In support of this conclusion the author draws arguments from the facts that hybrid offspring resulting from reciprocal crossings are often very different; that the offspring of a male hybrid and the female of a pure species is much more variable than the offspring of a female hybrid and the male of a pure species; that a structure which is more developed or of more functional importance in the male parent than it is in the female parent is very much more apt to vary in the offspring than a part which is more developed or more important in the mother than it is in the father. These and other facts convince Brooks that the ovum and sperm cell are not only different morphologically, but that they differ profoundly in function as well.

¹⁸ Professor H. V. Wilson, University of North Carolina.

In developing this idea into an explanatory theory of the way in which hereditary transmission is accomplished, Brooks borrows from Darwin's hypothesis of pangenesis, and assumes the existence of material particles, "gemmules," which are thrown off from the body cells. Unlike Darwin, however, he assumes that such particles are only thrown off at particular periods, when the body cells are disturbed in function through some change in their environment. The gemmules may penetrate an ovum or a bud, but it is the male germ cell which has gradually acquired during the evolution of the metazoa the peculiar power to gather and store up gemmules. The ovum on the other hand has acquired a very different nature. It contains material particles which correspond to the hereditary characteristics of the species. Thus in the case of a fertilized egg, as in that of a parthenogenetic egg, the great bulk of the development is due to the properties of the ovum itself. The gemmules brought in by the sperm cell unite with homologous particles in the ovum and so composite particles are produced which, as the egg segments and develops, give rise to cells that are strictly hybrids and which therefore exhibit variation. The ovum thus is the conservative element which transmits the characteristics that have already been acquired. The male cell is peculiarly that which stores up the disturbing effects of a changing environment. It especially leads, therefore, to variability in the offspring, to the production of individual differences.

This ingenious hypothesis enables Brooks to explain a great variety of inheritance phenomena and to overcome several serious objections to the unassisted selection theory. Whatever truth there may or may not be in the special ideas of the book, it remains to-day a stimulating and suggestive contribution, and it is properly looked on as one of the factors that have in recent years focussed the attention of the biological world on the problems of heredity.

Minor papers dealing with heredity and evolution, the causes of variation, and the determination of sex, appeared from time to time. Sections of the "Foundations of Zoölogy" (1899) show, too, that Brooks' interest in the questions discussed in the "Law of

Heredity'' remained active during life. Two of his last addresses (1906-1909) deal with our concepts of heredity and variation. In these he emphasizes the fact that the nature of an organism is not implicit in the egg, or in the organism indeed at any time of its life, but that it depends on a continuous reciprocal interaction between the organism and its environment. Such interaction leads in any particular case to a result which could not be calculated from a knowledge, however complete, of the egg itself since it is dependent not only on the organism but on the action of the total environment. The outcome of such interaction is the production of individuals which are never quite alike, although they may resemble one another closely. The occurrence of likenesses, or inheritance, and the occurrence of differences, variation, are thus not two processes but two views of the single process of reciprocal interaction. The idea that they are distinct is an error into which we fall through concentrating our attention at one time on the resemblances, and again on the differences between individuals. These considerations, he thinks, show the uselessness of theories which postulate an inheritance substance and explain individual differences as the result of various combinations of its particles.

These addresses show that Brooks has in some measure shifted his standpoint since the time of the "Law of Heredity." He no longer is in a mood to employ evolution (determinant) hypotheses to account for development. He now looks on the development of the individual, and that of races also, as epigenetic in nature. What will be the outcome of an individual egg depends on the interaction between egg and environment, not on a determinate mechanism in the egg. The pre-cambrian fauna has given rise to the living beings of to-day. But the latter were not implicit in the former, for with the same ancestors the course of evolution might have been different had the sum total of environmental influences been different.

*Writings on the Principles of Science.*¹⁹ Brooks dwelt often in conversation and in minor writings, and always with an earnest

¹⁹ Professor H V Wilson, University of North Carolina

pleasure, on the nature and intellectual value of what we can learn. His thoughts in this field of the principles of science were eventually embodied in his lectures on the "Foundations of Zoölogy" (1899). This remarkable book "belongs to literature, as well as to science. It belongs to philosophy as much as to either, for it is full of that fundamental wisdom about realities which alone is worthy of the name of philosophy."²⁰

Brooks was distinctly the philosophic type of naturalist. He was fully informed, critical, and constructive in special fields, but always aware that such fields were merely parts of a larger whole. Thus through the bent of his mind Brooks, the keen-sighted pioneer, and influential biologist, was also interested in and in thorough sympathy with life and living in all aspects, past, present and future, intellectual, emotional, and religious. Perhaps for that reason, too, he was a great teacher and inspirer of men.

The "Foundations" is essentially a discussion of the nature of scientific knowledge. It is the wise talk of an experienced, reflective naturalist of ripe years addressed primarily to younger fellow-workers in the fields of science. The argument which makes its way through pages and sometimes whole chapters of illustrations and digressions, interesting and suggestive in themselves, proceeds about as follows:

Our only knowledge of nature is through experience. Through experience we learn that one sort of event follows another, and this sequence, which we come to expect, constitutes for us the order of nature. Nevertheless there is no reason to believe that there is an inherent necessity in this order, for we never perceive the presence of any intrinsic causal connection between the preceding event (cause) and the succeeding one (effect).

When our knowledge of any part of nature has so far developed that we know the order of events, and so can predict the later steps in the series of occurrences, once the earlier have been noted, we say that we understand and can mechanically explain that particular set of phenomena. At present a gap separates vital

²⁰ President D. S. Jordan.

from non-vital phenomena—to say that life is the sum of the physical properties of protoplasm is to make a dogmatic assertion, although to gainsay it is to make another. But with the progress of science this gap may be bridged over at some time. Should it be bridged over, and life in all of its aspects be found to be “protoplasmic,” still we should not know *why* synthesis of compounds results in an organism or *why* a vital action is the outcome of protoplasmic changes. In respect to organisms and vital actions we should still be where we are now in respect to simple gravitation phenomena, for with respect to them all that we can say is that the stone will fall (if the future be like the past), but why it should fall we do not know.

This being the nature of our knowledge, present and future, what should the biologist seek to discover, and what are the problems that peculiarly concern him? Life is defined as a continuous adjustment of internal to external relations (Spencer), and it is pointed out that synthesized protoplasm, even were it capable of nutrition, growth, reproduction, and contraction, would not be a living thing if it were not also able to maintain persistent adjustment to the shifting world around it. The essence of the living thing and that which distinguishes it from other forms of matter is this very adjustment. Fitness, adaptive response, is therefore what we should seek to study in biology. The mechanism itself is of subordinate importance. Study it as we may, we cannot thus go far forwards, since our knowledge of nature never includes a perception of any necessary causal connection between events, such as would make it possible to discover vital phenomena by reasoning deductively from protoplasmic peculiarities. A corollary of practical import is that the naturalist should endeavor to study living things in connection with their environment.

Biology being thus defined as the study of adaptive response, the nature and evolution of man's reason and knowledge fall within its scope. For these are conceivably but the outcome of adaptive responses in the beginning as simple as the geotropism of a seedling's radicle. The ability, for instance, to make a distinction between what in practical life we call a truth, a real occurrence,

and an error or illusion, is to be looked on as a useful response that has been acquired through selection. Man's knowledge, then, is of the peculiar kind that is useful to him. He may not yet know as much as is good for him, but he at least has acquired a store of the kind of knowledge that preserves him in the struggle for existence.

Viewing man thus from the biological standpoint Brooks attempts to deal with two human characteristics, the consciousness that the will is free and that the individual carries a moral responsibility. These, like all other vital characteristics, he thinks, may possibly sometime be shown to be part of the order of nature and in that sense mechanical. "Rational action may sometime prove to be reflex from beginning to end." And yet in the face of this possibility, Brooks would still maintain that the will is free and moral responsibility real. To some this will seem a difficult thesis.

Underlying the scientific inquiry as to the character of our present knowledge and of that which possibly we may acquire about nature, is the metaphysical question, "what is nature?" This question Brooks does not attack in the fashion of constructive technical philosophy. He makes no attempt to define reality. His purpose in dealing with the matter is plainly the practical one of showing us what we need not believe. He says in effect, if then our knowledge of all nature is and will continue to be of one sort, viz., that phenomena follow one another regularly and (supposing the future to be like the past) in predictable fashion, but without our ever learning why they so follow one another, there is not now nor will there be in the future any necessity drawn from science to believe in a fixed, necessary, determinate nature. If in any quarter it is imagined that the progress of science necessitates or may necessitate such a belief, this is a grave error: in his own words, "The belief that the establishment of scientific conceptions of nature shows that after the first creative act, the Creator has remained subject, like a human legislator, to his own laws, is based upon utter misapprehension of science, and upon absurd and irrational notions of natural law." In the second place we are in no wise forced to believe by anything in science that

protoplasm and life are necessarily linked together: " . . . if it be admitted that we find in nature no reason why events should occur together except the fact that they do, is it not clear that we can give no reason why life and protoplasm should be associated except the fact that they are? And is it not equally clear that this is no reason why they may not exist separately?"

The next step in this survey and analysis of fundamental aspects of nature brings us to positive belief itself. As so often said, science quite fails to find in matter and motion any intrinsic virtue which sustains and directs the sequence of phenomena, and is absolutely restricted to the discovery of the mere sequence which itself calls for (metaphysical) explanation. Hence there is nothing in science which has any bearing on the causal origin or on the reality of anything in nature, and we must go elsewhere for the foundations of the belief that we may entertain in respect to such matters. Brooks believes that "nature is intended" to be as it is, and is a language which a rational being may read. Since the rational being is perhaps himself a part of nature's mechanism, this is equivalent to saying that one part of the mechanism is cognizant of the purpose that animates the whole. This purpose is the effect of a power, a sustaining and directing intelligence outside nature, to which both the origin of nature and its maintenance from day to day are due. It is not something which once for all set a determinate cosmos spinning along the path of time with a full complement of "eternal iron laws." It is something which is at work now, under every phenomenon. This is obviously Brooks' belief, although being no propagandist he is far from enforcing it, indeed leaves it in a measure to be inferred. What he wishes to make plain is that science does not tell us why events happen as we learn they do, and so it tells us nothing of ultimate reality. The question why the events we expect (from experience) should be those that come to pass concerns not science but "the natural theologian; for it is the same as the question, What is the Cause of Nature? To this all must seek an answer for themselves; for each has at his command all the data within the reach of any student of science."

LIST OF PROFESSOR BROOKS' WRITINGS²¹

1864

Do animals reason? Wilkes' Spirit of the Times.

1874

A feather. Pop. Sci. Monthly, April, 1874.

1875

On an organ of special sense in the lamellibranchiate genus *Yoldia*. Proc. American Asso. Adv. Sci., Hartford meeting, August, 1874, 3 pp. 2 cuts. Printed Salem, 1875.

Embryology of *Salpa*. Proc. Boston Soc. Nat. Hist., vol. 18, November 17, 1875, 7 pp., 1 pl.; also Monthly Microscopical Journal, London, July 1, 1876.

1876

Embryology of the fresh-water mussels. Proc. American Asso. Adv. Sci., Detroit meeting, 1875, 3 pp. Printed Salem, June, 1876.

A remarkable life-history and its meaning. American Naturalist, November, 1876, 16 pp. 17 cuts.

On the development of *Salpa*. Bull. Mus. Comp. Zool., vol. 3, March, 1876.

On the affinity of the Mollusca and Molluscoida. Proc. Boston Soc. Nat. Hist., vol. 18, February 12, 1876, pp. 225-235.

1877

A provisional hypothesis of pangenesis. American Naturalist, March, 1877, 4 pp. (Abstract of paper read at Buffalo meeting. American Asso. Adv. Sci., August, 1876).

Parthenogenesis in vertebrates and molluscs. American Naturalist, October, 1877.

Instinct and intelligence. Pop. Sci. Monthly, September, 1877.

1878

Differences between animals and plants. Pop. Sci. Monthly, November, 1878.
The condition of women from a zoological point of view. Pop. Sci. Monthly, June and July, 1879.

²¹ Compiled by Prof. E. G. Conklin. Professor Brooks made no list of his publications and the following list has been compiled from many sources and may not be entirely complete.

1879

The scientific results of the Chesapeake Zoölogical Laboratory, session of 1878. Baltimore, Murphy, 1879, 168 pp., constituting part 1, vol. 1, Studies Biological Laboratory, Johns Hopkins University, containing the three following papers by W. K. Brooks:

Preliminary observations upon the development of the marine prosobranchiate gasteropods; 47 pp., 1 pl.

The development of *Lingula* and the systematic position of the Brachiopoda; 70 pp., 6 pls.

The larval stages of *Squilla empusa* Say; 5 pls.

The development of the digestive tract in molluscs. Proc. Boston Soc. Nat. Hist., vol. 19, 1879, 4 pp.

Abstract of observations on the development of the American oyster. Zool. Anzeiger, 1879.

Abstract of observations upon the artificial fertilization of oyster eggs, and on the embryology of the American oyster. American Journ. Sci., December, 1879. 3 pp.

Observations upon the early stages in the development of the freshwater pulmonates. Studies Biol. Lab. Johns Hopkins University, vol. 1, 1879. 26 pp., 4 pls.

1880

Observations upon the artificial fertilization of oyster eggs and on the embryology of the American oyster. Ann. and Mag. Nat. Hist., London, 1880.

The biology of the American oyster. N. C. Med. Press, 1880.

The artificial fertilization of oyster eggs and the propagation of the American oyster. American Journ. Sci., 1880.

The development of the American oyster. Maryland Fish Commission Report, 1880, 101 pp., 10 pls.

The development of the oyster. Studies Biol. Lab., Johns Hopkins University, vol. 1, 1880, 115 pp., 11 pls. (Reprinted from the preceding.)

The acquisition and loss of a food yolk in molluscan eggs. Studies Biol. Lab., Johns Hopkins University, 1880, 7 pp., 1 pl.

Budding in free Medusæ. American Naturalist, 1880.

Embryology and metamorphosis of the Sergestidæ. Zool. Anzeiger, Jahrg. 3, 1880.

Amphioxus and *Lingula* at the mouth of the Chesapeake Bay. American Naturalist, vol. 13, 1880.

The young of the crustacean *Lucifer*, a Nauplius. American Naturalist, November, 1880.

The development of the squid. Anniversary Mem. Boston Soc. Nat. Hist., 1880, 21 pp., 3 pls., 40.

The homology of the cephalopod siphon and arms. American Journ. Sci., vol. 20, October, 1880, 3 pp., 1 cut.

The rhythmical character of the process of segmentation. American Journ. Sci., vol. 20, 1880, p. 293.

1881

- Du développement de la lingula et de la position zoologique des brachiopods. Arch. de Zool. Exp. et Générale, 1881.
- Lucifer, : A study in morphology. Proc. Royal Soc., April, 1881, pp. 1-3. (Abstract.)

1882

- Origin of the eggs of Salpa. Biol. Studies, Johns Hopkins University, vol. 2, 1882, pp. 301-312, 1 pl.
- Lucifer: A study in morphology. Phil. Trans. Royal Society, London, vol. 173, 1882, 80 pp., 11 pls.
- Handbook of invertebrate zoology 400 pp., 202 figs. Boston, Cassino, 1882.
- Chamisso and the discovery of alternation of generations Zool. Anzeiger, Jahrg. 5, 1882.
- The metamorphosis of Alpheus Johns Hopkins University Circulars, vol. 2, no. 17, 1882.
- On the origin of alternation of generations in Hydro-medusæ. Johns Hopkins University Circulars, vol. 2, no. 22, 1882; also Ann. and Mag. Nat. Hist., vol. 2, 1883
- The metamorphosis of Penæus. Johns Hopkins University Circulars, vol. 2, 1882; also Ann. and Mag. Nat. Hist., vol. 2, 1883
- Speculative zoology Pop. Sci. Monthly, December, 1882, and January, 1883.
- On some methods of locomotion in animals A lecture delivered to the employés of the Baltimore and Ohio Railroad Co., Baltimore Printed by I. Friedenwald for free distribution among the employés of the Baltimore and Ohio Railroad Co., 20 pp., 10 cuts, 1882

1883

- List of Medusæ found at Beaufort, N. C., during summers of 1880-1881. Studies Biol. Lab. Johns Hopkins University, vol. 2, 1883, pp. 135-146
- Report of the Chesapeake Zoological Laboratory, summer of 1882
- The law of heredity. Baltimore, Murphy, 1883, 336 pp
- Notes on the Medusæ of Beaufort, N. C., II. Turritopsis nutricula (McCrary). Studies Biol. Lab. Johns Hopkins University, vol. 2, 1883, pp. 465-475.
- The first zoœa of Porcellana. Studies Biol. Lab. Johns Hopkins University, vol. 2, pp. 58-62, pls. 6-7. (With E. B. Wilson.)
- Alternation of periods of rest with periods of activity in the segmentation of eggs of vertebrates Studies Biol. Lab. Johns Hopkins University, vol. 2, 1883, pp. 117-118.
- The phylogeny of the higher Crustacea Science, vol. 2, pp. 790-793; also New Zealand Journal of Science, vol. 2
- Reviews of work on cœlenterates in Weekly Summary of the Progress of Science. Science, vol. 1, pp. 50, 81, 230, 287, 344, and 553; Science, vol. 2, pp. 54, 692, 773, and 832.

1884

- Is *Salpa* an example of alternation of generations? *Nature*, vol. 30, 1884.
- On the life-history of *Eutima* and on radial and bilateral symmetry in hydroids
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STUDIES ON CHROMOSOMES

VI. A NEW TYPE OF CHROMOSOME COMBINATION IN METAPODIUS

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WITH FIVE FIGURES

Although the peculiar combination of chromosomes here to be described has been seen in only a single individual, it affords new and I think significant evidence regarding some of the most interesting of the problems connected with the nuclear organization. As was shown in the fifth of my "Studies on Chromosomes,"¹ the genus *Metapodius* is most exceptional and remarkable in that the specific number of chromosomes varies, while that of the individual is on the whole constant. It is true that slight indiscriminate fluctuations in the number of the ordinary chromosomes, or "autosomes," occur, as they do in many other species; but this is only an inconsiderable source of the specific variation. The evidence shows, beyond a doubt in some individuals, and hence with probability for all, that the numerical differences are primarily due to variations in the number of a particular class of chromosomes which I called the "supernumeraries." These may be wholly absent. When present, their number is constant in the individual, but differs in different individuals. They are often recognizable in both sexes by their size, and in the male also by certain very definite peculiarities of behavior in the maturation-process. When they are absent, the diploid groups contain 22 chromosomes; and this condition is almost certainly the fundamental type of the genus, of which all the other conditions are variants. Such a group comprises 18 ordinary chromosomes, or "autosomes" + 2 very small microchromosomes, or *m*-chromosomes + 2 unequal idiochromosomes = 22 (these respective

¹ Wilson: '09c.

classes having the peculiarities heretofore described).² Numbers above 22 arise through the addition of one or more relatively small "supernumeraries," which agree in behavior with the small idiochromosome, of which they are probably duplicates. None of my own material (53 individuals, of three species) showed less than 22 chromosomes, and at least one small idiochromosome was present in all. In all of Montgomery's material of *M. terminalis*, however (9 individuals), this chromosome is absent, the spermatogonial number is but 21, and the large idiochromosome appears without a synaptic mate as a typical odd or accessory chromosome.

The foregoing results were based on the study of 62 individuals in all, representing the three species, *terminalis*, *femoratus* and *granulosus*. In February, 1909, I took at Miami, Fla., two additional male specimens of *femoratus*, quite typical in structure, and closely similar in external appearance. One of these (No. 63) is an ordinary 23-chromosome form with one large supernumerary (like Nos. 13 or 48 of the general list given in "Study V") and is only of interest for comparison with the other individual. The latter, hereinafter designated as "No. 64," shows a different chromosome-combination from any heretofore seen in this genus or elsewhere. The diploid groups (spermatogonia) contain 22 chromosomes; but both these groups in themselves and their history in maturation proves most clearly that they are not the same as in the typical 22-chromosome forms, differing from the latter in respect to the idiochromosomes and the *m*-chromosomes. In the typical forms there are, as stated above, two of each of these chromosomes. In No. 64, on the other hand, there are three *m*-chromosomes and but one idiochromosome (the large), the latter appearing as a typical odd or accessory chromosome, as in the material of Montgomery; thus, 18 autosomes + 3 *m*-chromosomes + 1 odd chromosome = 22. That this is the true interpretation of the facts is demonstrated by the behavior of these respective chromosomes in the maturation-process. I would emphasize the fortunate fact that both testes of the animal show excellent fixation and staining (strong Flemming, iron haematoxylin) and that they contain multitudes of division-figures which demonstrate

² Ibid: '05b, '05c, '06, etc.

all the stages. The agreement of great numbers of division-figures from both testes leaves no doubt regarding the constancy of the essential phenomena (with rare minor variations, as indicated beyond). As will be seen, the modification of the diploid groups has led to corresponding modifications of the maturation-process that are most interesting in relation to some of the problems of synapsis and of the qualitative differences of the chromosomes.

DESCRIPTIVE

a. The spermatogonial groups

The peculiar anomaly of the chromosome groups, first seen in the spermatocyte-divisions, led me to examine the spermatogonial groups with particular care, and it will be worth while to state both the preliminary and the definitive results. These groups are in the nature of the case more difficult than those of the spermatocytes, owing to the greater number, smaller size, and greater crowding of the chromosomes; hence, only flat metaphase-plates and such as are not very oblique to the plane of section can safely be used. A search through the numerous dividing spermatogonia showed 35 cases that seemed to meet these conditions and also to show no serious obscurity or confusion of the chromosomes. Many of these are of almost schematic clearness, and some are well adapted for photographic reproduction. The first examination showed undoubtedly that 29 of the 35 cases contained 22 chromosomes each, including 19 large and three very small ones. Of the six exceptions, three seemed to lack one of the small ones, two, one of the large ones, and one a large and a small. Close study of these six cases ultimately showed that in four cases the apparently missing third small chromosome was in reality present, though hidden among the larger chromosomes, while in two cases an apparently missing larger chromosome was found lying immediately below another one, the metaphase-plate not yet having become perfectly flat. This leaves but one exception in 35 cases, and we shall hardly go astray in the conclusion that this exception is probably the result of accident. In any case we may confidently conclude that the chromosome-group shown in

the 34 cases may be taken as characteristic of the dividing spermatogonia, and that it occurs with a high degree of constancy.

Six of these groups, from the best that could be found, three from each testis, are shown in fig. 1, *a-f*. These have been selected particularly to show the different positions of the three small chromosomes. The latter appear to follow no rule whatever, the three lying anywhere in the metaphase-plate; and all may be separate, all together, or two together and one separate. This is an interesting and significant fact, because in the first spermatocyte-division, as described beyond, the three are always associated to form a triad element which invariably occupies the same position in the chromosome-group (see p. 58).

For the sake of comparison, four spermatogonial groups from other individuals are here reproduced (from my fifth Study). Two of these (fig. 1, *i, j*) are from femoratus, No. 29, which has the typical diploid group of 22 chromosomes, including but two small (*m*-chromosomes.) The other two (fig. 1, *g, h*) are from terminalis, No. 2, which has 23 chromosomes, including two *m*-chromosomes and one small supernumerary³. As will appear beyond, this latter chromosome is wholly different in nature from the third small chromosome in individual No. 64, though indistinguishable from it by the eye in the spermatogonial groups.

As the figures show, the larger chromosomes in No. 64 show well marked size-differences, and in most of the groups a largest and second largest pair are usually fairly evident; but it is impossible to pair all of the chromosomes accurately by the eye. It is, however, obvious that not more than 18 of the 19 can be equally paired. One of them must either have no proper mate, or it must form a very unequal pair with the third small chromosome. The following possibilities must, accordingly, be considered:

1. The nineteenth large chromosome and the third small one are respectively a large and an abnormally small idiochromosome which form a pair of synaptic mates, or

2. The nineteenth large chromosome is an odd or accessory chromosome, without a synaptic mate, while the third small one is similar to a small "supernumerary" or

³ Cf: Photo. 29, Study V.

All the figures are from camera lucida drawings. With a few exceptions they are a little more enlarged than those of Study V.

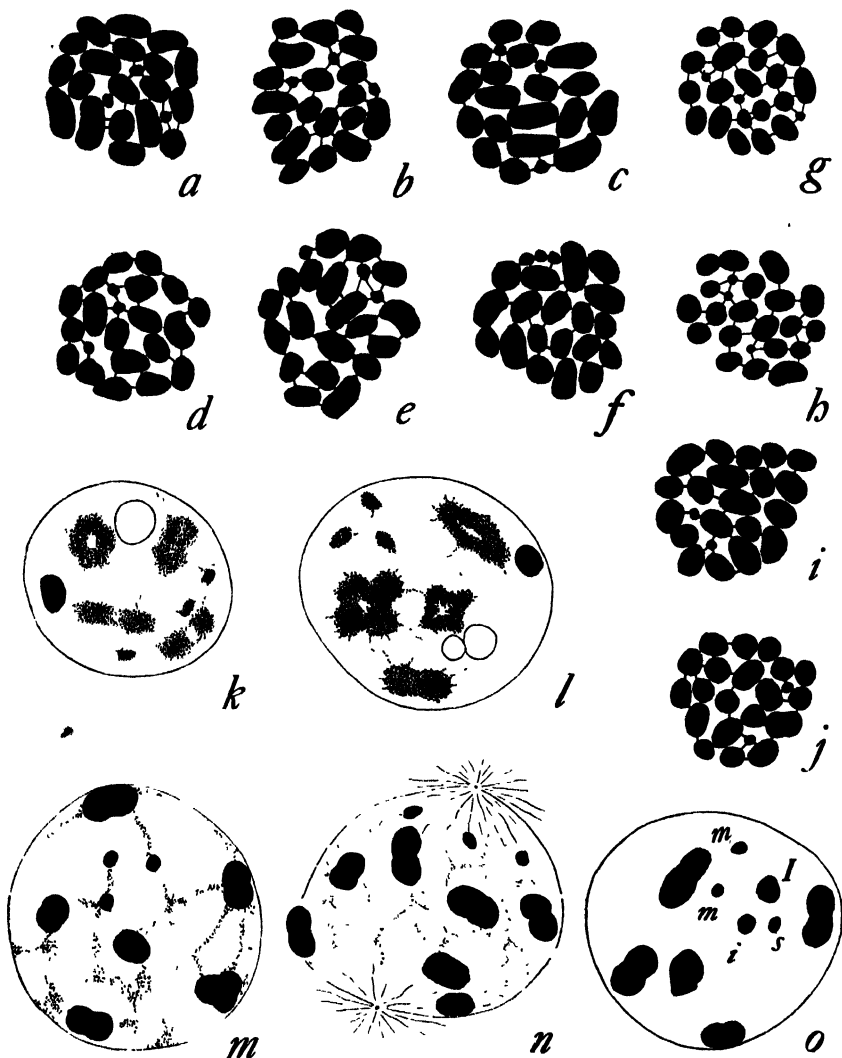


Fig. 1 *a-f*, spermatogonial groups. *M. femoratus*, No. 64, three from each testis; *g, h*, spermatogonial groups for comparison from *M. terminalis*, No. 2, with one small supernumerary (23 chromosomes); *i, j*, spermatogonial groups from *M. femoratus*, No. 29 (22 chromosomes); *k, l*, early prophases, No. 64; *m, n*, late prophase of same; *o*, late prophase for comparison, from *M. terminalis*, No. 43, with one small supernumerary (23 chromosomes)

3. An odd or accessory chromosome is present, and also a third *m*-chromosome.

A study of the maturation process decisively establishes the third of these possibilities as the fact.

b. The first spermatocyte-division

Both testes contain immense numbers of both spermatocyte-divisions in all stages, and many of the cysts show the facts with great beauty. The first division itself at once indicates the true interpretation of the spermatogonial groups; and this is consistently borne out by the stages which precede and follow.

In polar views (fig. 2, *d-g*) the first division metaphase is identical in appearance with that of *Anasa*, *Chelinidea*, *Narnia*, and other coreids that have 21 spermatogonial chromosomes (including Montgomery's individuals of *M. terminalis*). Eleven chromosomes appear, including one very small central one surrounded by a ring of nine much larger ones, while the eleventh usually occupies a position outside the ring, as in figs. 2*d*, 2*f*, (figs. 2*e*, 2*g* are given to show exceptions to this). From these views alone we should infer that the spermatogonial number is 21, that the small central chromosome is the *m*-bivalent, and that the eccentric one is the accessory. This will appear upon comparison with figs. 2, *h*, *i*, which show two corresponding views of Montgomery's material of *M. terminalis*. Side views at once reveal the fact, however, that the central body in No. 64 is not a bivalent but a triad element, consisting of three small chromosomes united end to end (figs. 2*b*, 3*a*, *b*.) and it is perfectly evident that these are identical with the three very small ones of the spermatogonial groups. Hundreds of these figures have been observed, in almost all of which the three components have the linear arrangement just described; but now and then a different grouping occurs, as may be seen in both side (fig. 3*c*) and polar views (fig. 2*g*).

In the ensuing division the ten larger chromosomes divide equally, showing as they draw apart the curious forms represented in fig. 4, which are closely similar to those described in *Anasa* by Paulmier ('99). As the figures show, the chromosomes in

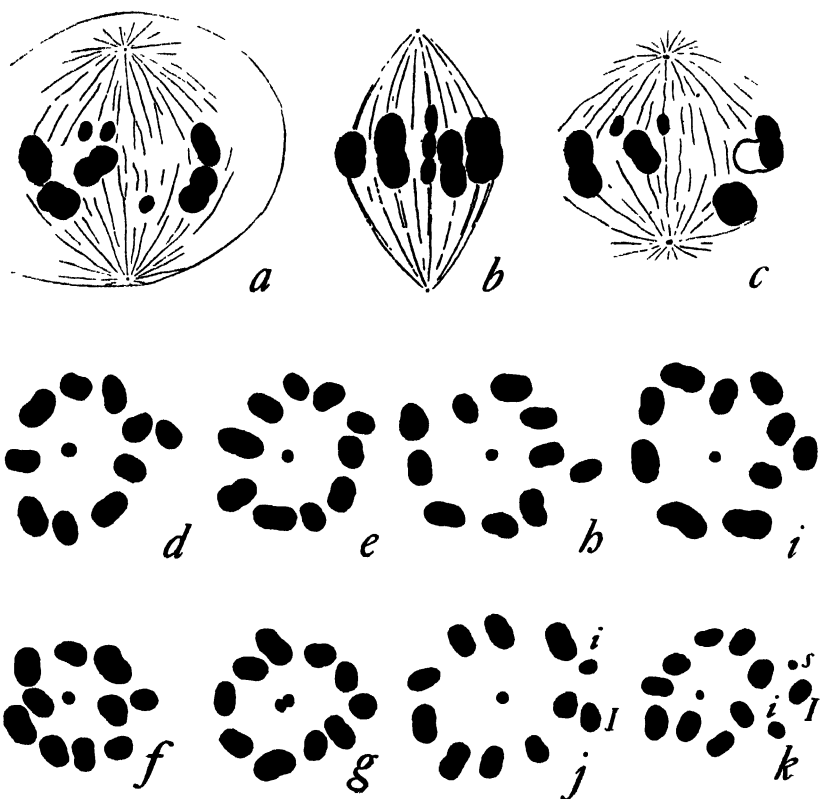


Fig. 2 *a*, late prophase No. 64, spindle forming; *b*, metaphase of same in side view; *c*, late prophase of *M. femoratus*, No. 29 (22 chromosomes), for comparison with *a*; *d-g*, first division metaphase, polar views, No. 64; *h, i*, similar views of *M. terminalis*, No. 3 (Montgomery's material), with 21 chromosomes; *j*, similar view of *M. femoratus*, No. 29 (22 chromosomes); *k*, similar view of *M. terminalis*, No. 43 (23 chromosomes), with one small supernumerary, for comparison.

the early anaphase are more or less clearly quadripartite, and separate into bipartite daughter chromosomes connected by conspicuous double fibers (figs. 4, *b-h*) but true tetrads (such for instance, as those observed by Levfere and McGill in *Anax*, '08) are rarely if ever seen. The quadripartite form, though very characteristic of this division, is by no means invariable in case of the large bivalents, and has not been seen in case of the eccentric odd chromosome.

In the mean time the small central triad breaks up into its separate components, which then pass to the poles in a very interesting fashion. This process always begins before the division of the large chromosomes, and is subject to some variation. Most frequently the three components draw apart in such a way as to leave the middle one lagging near the equator of the spindle while the others are proceeding towards the poles (figs. 3*h*, *i*). Often, however, one component first separates from the other two (figs. 3*j*, *k*); but even in this case it seems probable that one of the latter is afterwards left lagging on the spindle, since later in the anaphases this arrangement is almost invariable. In these stages the middle component frequently becomes drawn out along the spindle to form a rod which finally passes to one pole to enter the telophase group (figs. 4*e*, *f*). Half the secondary spermatocytes thus receive two small chromosomes and half but one, the respective numbers being 12 and 11.

Two observed anomalies may briefly be mentioned. In two or three cases the middle component seems to be degenerating on the spindle (fig. 4*g*); but if this be really the case it must be of rare occurrence, as is shown by the second division. Another interesting anomaly is shown in fig. 4*h*. Here there are apparently five small chromosomes, two of which are smaller than the others and are connected by a fiber as if they had recently divided. I am uncertain how to interpret this case, for one of the larger chromosomes (stippled in the figure) is paler than the others and lies at a lower level. This may be a fragment of the original plasmosome. If this be the case we have before us a case in which the central small chromosome has divided precociously. If all the five bodies, on the other hand be chromosomes, one of them would seem to be an extra or adventitious body, comparable to those described and figured by Paulmier in *Anasa* ('99, fig. 28*a*).

c. The second spermatocyte-division

As is to be expected from the asymmetrical distribution of the three small chromosomes in the first division the secondary spermatocytes are of two classes. These divisions are very numerous

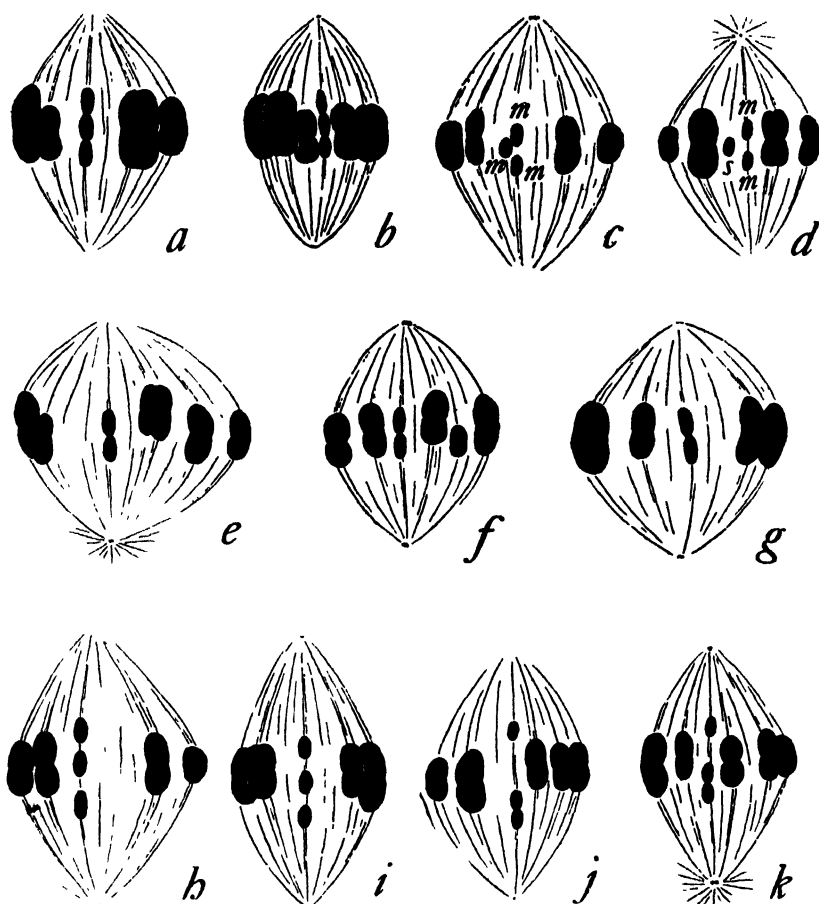


Fig. 3 Metaphases and anaphases of the first division, No. 64, in side view. *a*, *b*, typical side views, with linear central *m*-triad; *c*, unusual grouping; *d*, similar view of *M. terminalis*, No. 1, for comparison, with one small supernumerary and *m*-bivalent (23 chromosomes); *e*, *g*, similar views of *M. femoratus*, No. 29 (22 chromosomes) for comparison; *f*, the same, *M. femoratus*, No. 46 (22 chromosomes); *h-k*, No. 64, initial anaphase, separation of the *m*-triad.

in both testes, and all the stages are shown by hundreds. In polar views of the metaphases about half the cells are seen to contain 11 chromosomes (fig. 5*a*, *b*) and half 12 (fig. 5*c*, *d*), the former containing but one small chromosome and the latter two.

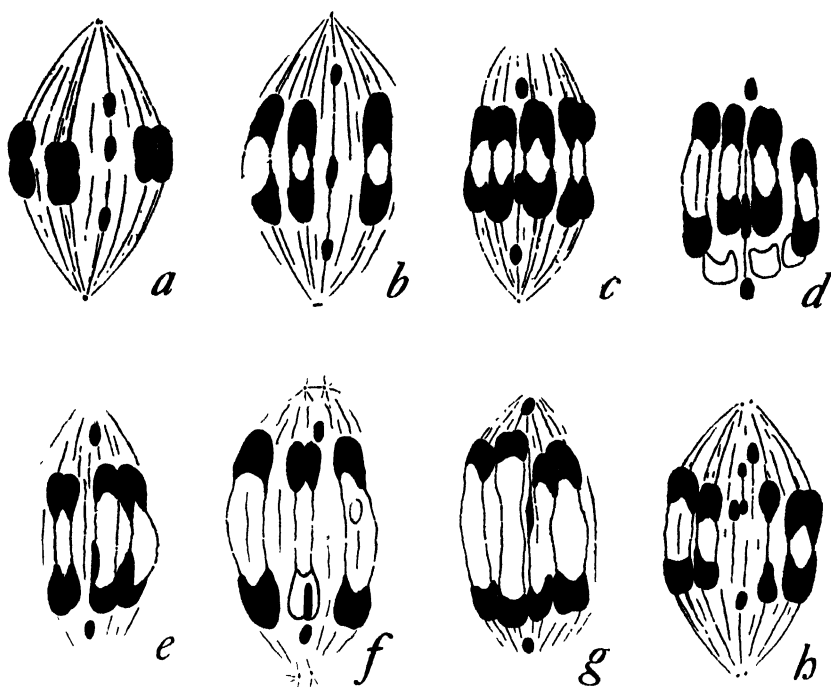


Fig. 4 Anaphases of first division, all from No. 64; *g* and *h* are atypical conditions.

As is the rule throughout the Coreidæ, the regular grouping characteristic of the first division is usually lost or obscured in the second. As a rule the ring formation is no longer seen, there is, no constantly eccentric chromosome, while the *m*-chromosome, invariably central in position in the first division, now occupies any position, though it is more frequently near the center of the group.

In side views of the metaphases all of these chromosomes, with one important exception, are dumb-bell shaped, and in the initial anaphases are seen drawing apart into a pair of daughter-chromosomes (fig. 5*e-g*). One chromosome, almost invariably *central* in position, forms an exception in showing no sign of constriction, its form being evenly rounded and often nearly spheroidal. As

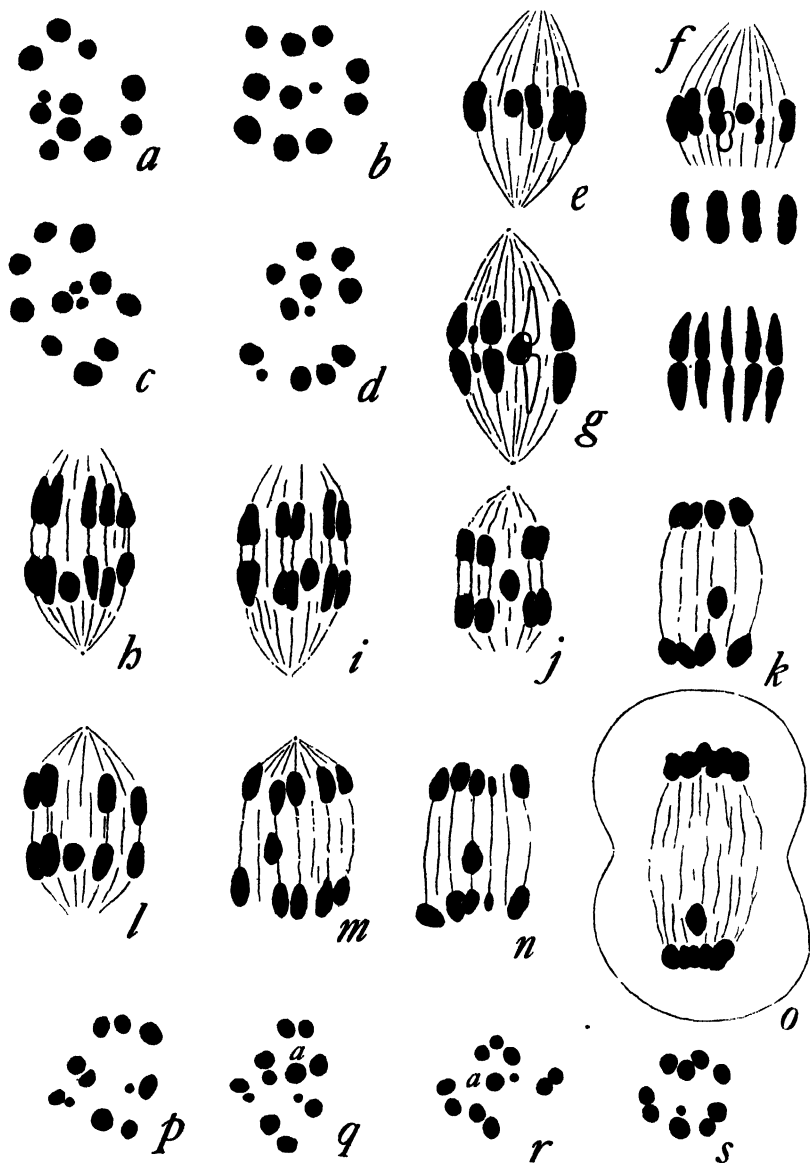


Fig. 5 Second division, No. 64. *a, b*, metaphases, polar views, 11 chromosomes; *c, d*, the same, 12 chromosomes; *e*, side view; *f*, the same showing all the chromosomes (four from lower level shown below); *g*, initial anaphase, all the chromosomes shown (five of them from a lower level at right); *h-o*, later anaphases; *p, q*, sister groups, from the same spindle, late anaphase, *p*, the upper group with 11 chromosomes; *q*, the lower group with 12; *r, s*, two late anaphase groups (not from the same spindle) to show the third and fourth types of spermatid nuclei.

seen in side views of the late metaphases or earliest anaphases (fig. 5g) this chromosome always appears darker and more conspicuous than the others (probably because it is not drawn out along the spindle fibers) and owing to this circumstance its history during these stages may be followed with an ease and certainty of which the figures give but an imperfect idea. As the bipartite chromosomes separate in the anaphases the chromosome in question is usually left lagging near the equator of the spindle, though not infrequently it lies in one of the daughter groups (figs. 5, *h-n*).

In the late anaphases, as the cell-body is dividing, it may be seen passing, without constriction, diminution in size, or other sign of division, into one of the daughter-nuclei (fig. 50). I desire especially to emphasize the fact that these processes are seen with such clearness and in so great a number of cells, as to leave not the remotest doubt that this chromosome neither divides nor separates from an accompanying mate. It is therefore a typical odd or accessory chromosome, or unpaired idiochromosome, identical in its general history with that seen in *Anasa*, *Protenor*, and so many other forms. In the second maturation-division, accordingly, one of the daughter-cells in each case receives one chromosome less than the other; and since there are two classes of secondary spermatocytes, there are four classes of spermatids and of spermatozoa. All receive nine ordinary chromosomes and one *m*-chromosome. Two-fourths contain and two-fourths lack a second small chromosome; and each of these two-fourths falls into two classes, one containing and one lacking the accessory chromosome. These four classes are readily distinguishable in polar views of rather late anaphases, particularly in cases where the accessory chromosome lies at the same level as the chromosomes of one daughter group. Fig. 5*p,q* show two such daughter groups, from the same spindle and in the same section. In each case two small chromosomes are present, and one group contains 11 chromosomes, the other 12. I could not find a single case of the other type (with 10 and 11 chromosomes) in which both daughter-groups appear in the same spindle: but two anaphase groups from different spindles are shown in fig. 5*r, s*, the former containing 11 chromosomes, the latter 10.

d. The Growth-period and Maturation-prophases

The foregoing facts demonstrate in the clearest manner that this individual of *M. femoratus* differs from all other individuals of the genus heretofore examined, with the exception of Montgomery's material of *M. terminalis*, in having an odd or unpaired idiochromosome (accessory chromosome) which corresponds to the larger member of the pair of unequal idiochromosomes found in other individuals. They show also that the third small chromosome is not a small supernumerary of the type found in other individuals, and is nothing other than a third *m*-chromosome. This is fully borne out by the growth-period and prophases. As I have indicated in earlier papers, the *m*-chromosomes are in general characterized during the growth-period by the fact that they remain univalent (there are some exceptions to this) and in most cases (of which *Metapodius* is one) are in a diffuse and light-staining condition. Further, as was first shown by Gross ('04) in *Syromastes*, as a rule they only conjugate to form a bivalent in the final prophases of the first division—very often not until the spindle is formed and the chromosomes are entering the equatorial plate. Such a late prophase, from a 22-chromosome individual of the same species (No. 29) is shown for the sake of comparison in fig. 2*c*, the two separate *m*-chromosomes appearing above and towards the left. Their final conjugation always takes place at the center of the group (fig. 2*j*, 3, *e-g*).

In individual No. 64, prophases of every stage are shown in hundreds of nuclei. In the latest stages, after the nuclear wall has broken down, three separate small chromosomes are shown (fig. 2*a*) which may be seen coming together in the final prophases to form the small central triad. Figs. 1*m*, *n* show two earlier stages from the same cyst with the last, one of them showing the beginning of the spindle-formation, the other an earlier stage when the asters are very small and often invisible. Each of these shows the three separate small chromosomes, as before. At this time all the chromosomes are compact and deeply stained. In still earlier stages, at a time when the bivalents are all diffuse and appear in the form of lightly staining double crosses, rods, etc.,

the three small chromosomes are still easily seen in many of the nuclei; but they are now pale and diffuse like the bivalents. In this respect the third small chromosome differs from a "super-numerary" of the type described in my former paper, and agrees exactly with the *m*-chromosomes.

Each nucleus contains at this period a single compact, rounded and intensely staining chromatic nucleolus, which is no doubt the odd or accessory chromosome (monosome), as in so many other forms,⁴ and in addition there is present a conspicuous, rounded

⁴ This identification is in agreement with that of most observers in recent years. A few writers have however disputed the view that the chromatic nucleolus of the growth period of the spermatocytes is a chromosome—*e. g.*, Moore and Robinson in case of the cockroach ('05), Foot and Strobell in the case of *Anasa* ('07) and *Euschistus* ('09), and Arnold ('08), in case of *Hydrophilus*. The results of Moore and Robinson on this point are opposed by those of Stevens ('05), Wassilieff ('07), and more particularly by the detailed observations of Morse ('09). Those of Foot and Strobell on *Anasa* are not sustained by the later ones of Lefevre and McGill ('08). Among others who have in the past two years adhered to the view here adopted may be mentioned Otte ('07), Davis ('08), Boring ('07), Jordan ('08), Stevens ('08, '09), McClung ('08), Robertson ('08), Randolph ('08), Nowlin ('08), Payne ('09), Wilson ('09b, '09c), Guthertz ('09), Wallace ('09), Gerard ('09), and Buchner ('09a). Since I intend to return to the subject hereafter I will take this occasion for only brief comment on some of these results, without attempting a full review of the literature.

Moore and Robinson, who have been followed by Arnold (Strasburger, '07, '09. expresses the same opinion) also regard the body that is seen passing to one pole in one of the maturation divisions ("accessory chromosome") as not a chromosome but a "nucleolus." I find it incredible that anyone can hold to such a view who reckons squarely with the large existing body of direct and detailed observation upon the accessory chromosome itself; and this view seems to be quite ruled out of court by comparative studies on the sex-chromosomes, such for instance as those of Payne on *Gelastocoris* and the reduvioids. I will not enter here upon the maze of difficulties regarding the numerical relations of the chromosomes which the same view involves, since they have already been indicated by Guthertz ('09), in a recent reply to Strasburger. My own preparations, including an extensive series of sections and smears especially of *Protenor*, *Lygæus* and *Pyrrhocoris* leave in my mind not the least doubt of the identity of the chromatin-(chromosome)-nucleolus of the growth period with the odd chromosome (monosome) of the spermatogonia, and with the heterotropic or accessory chromosome of the maturation-divisions.

Certain writers have seemed to take it for granted that the accessory chromosome or "monosome" is always characterized by its nucleolus-like condition in the resting nuclei, not only in the spermatocytes but also in the spermatogonial and other

pale plasmosome which is considerably larger than the chromosome nucleolus. This body, particularly well shown in these slides, is at once recognizable by its smooth contour, spheroidal form (sometimes double, as in fig. 11) and pale yellowish color after the hæmatoxylin, and it forms a striking contrast to the intense blue-black of the chromosome-nucleolus. Nuclei in which all five bodies—the three small chromosomes and both

diploid nuclei. This assumption—which is doing much to confuse the whole subject—may accord with the facts in certain species, but certainly is not generally true. Much of the recent work in this field, as well as some of the earlier (*e. g.*, that of McClung '00, and Sutton '00) goes to show that in many species it is only in the growth-period of the *spermatocytes* that this chromosome forms a chromosome nucleolus, not in the diploid nuclei of either sex. Such seems to be the case in all the Hemiptera that I have studied. In these animals the accessory chromosome, or its homologue the large idiochromosome, first assumes the nucleolus-like condition in the post-spermatogonial stages, when its origin from an elongate chromosome may in some species readily be followed step by step, as I have shown in *Lygæus* ('05b) and *Pyrrhocoris* ('09b). In the spermatogonia of these animals this chromosome does not differ visibly in behavior from the others and cannot be seen in the resting nuclei.

Several years ago, in two successive papers ('05a, '06) I described and commented on the interesting fact that in the female this chromosome (and its fellow, when present) seems in some species not to assume a nucleolus-like condition in the synaptic stage and early growth-period of the oöcytes. Since some doubts on this point were raised in my own mind by the later work of Stevens ('06) and Gutherz ('07) I am now glad to have the very positive confirmation of my results given by the work of Foot and Strobell ('09) on *Euschistus* (one of several forms I had examined). This confirmation must have been made without knowledge of my previous work, since the latter is referred to in neither text nor literature list, and the supposedly new facts are made the main basis for renewed attack upon my general conclusions. On the other hand Buchner ('09a) has recently found in the synaptic or "bouquet" stage of the oöcytes in *Gryllus* a nucleolus-like "accessory body" which he believes to be of the same nature as the accessory chromosome of the male, though its history in maturation was not followed out, nor is other proof of the conclusion given.

It is of some psychological interest to find Buchner on the one hand and Foot and Strobell on the other disputing my conclusions regarding sex-production on diametrically opposing grounds, the *first-named author because (as he believes) a chromosome-nucleolus is present in the oöcyte-nucleus, the last named because it is absent(!)*. In what way either of the mutually contradictory arguments invalidates or weakens my conclusions I am not yet able to perceive, nor need we here consider the contradiction in the data; but it is interesting to observe how each of the arguments goes awry by reason of the confusion regarding the chromosome-nucleolus, referred to above. Foot and Strobell, for example, argue that because

nucleoli—are visible in the same section are not very common. Two such cases are shown in figs. 1*k*, 1*l*, each of which shows also four of the nine larger bivalents.

I have not endeavored to make an exhaustive study of the growth-period as a whole, but the facts reported above taken in

such a body is not present in the oöcyte-nucleus, therefore the odd or accessory chromosome of the male cannot be derived in fertilization from the egg-nucleus—an obvious *non sequitur*. Buchner's argument, based upon precisely opposite data, shows a somewhat similar, though less obvious entanglement. The essence of his objection is given in the following passage, which at the outset accepts all the essential facts on which the conclusions of Stevens and myself were based. "Auf alle Fälle haben wir nur eine Sorte von Eiern, denn dass er (the accessory chromosome) in einem Ei ausgestossen und im andern innenbehalten wird, erscheint undenkbar. Die Spermatozoen haben das accessorische Chromosom zur Hälfte. Nehmen wir an, die Eier besäßen das accessorische Chromosom schon, so gäbe es Tiere mit zwei Monosomen und solche mit einem—*ein Fall der nicht existiert*" (*op. cit.*, p. 409, italics mine). This is, indeed, an astounding statement; for it was the very fact that there *are* individuals that have but one monosome or accessory chromosome (the males), and other individuals of the same species (the females) that have two corresponding chromosomes, upon which the conclusions of Stevens and myself were mainly based(!). This is true, as Gutherz ('08) has shown, of the very form (*Gryllus*) of which Buchner is writing, the single odd chromosome (monosome) of the male, recognizable by its peculiar form and other characters, being represented in the female by two such chromosomes. This is also in agreement with the results of other recent workers on the Orthoptera including Wassilieff, Davis, Jordan and Morse. I can therefore find no meaning in Buchner's statement unless the word "Monosom" be used to denote simply a chromosome-nucleolus, when the passage becomes at least intelligible. But such a restriction in the meaning of this word is not justified by its etymology, by the original definition of its author (Montgomery, '06*a*, '06*b*) nor by the facts; and it does not seem to accord even with Buchner's own usage elsewhere in the paper. That Buchner's statement is totally at variance with the facts when correctly stated is shown by the following summary of my results, quoted from one of the papers in which Montgomery first defined the word "monosome." "When there is a single monosome in the spermatogenesis (as in *Protenor*, *Harmostes*, *Anasa* and *Alydus*) there are *two* in the *ovogenesis* so that the ovogonia possess always an even number of chromosomes" ('06*b*, p. 145, italics mine).

But even if we admit that the "accessory body" of the female is a chromosome—and not only is there no proof of this but many reasons for doubting it—what adverse bearing would the fact have upon the "theory"? None as far as I can see, unless this chromosome were proved to be univalent and without a synaptic mate. Were all this true, new and unintelligible complications would arise in regard to the numerical relations of the diploid and haploid chromosome-groups in both sexes; but it is not worth while to consider these puzzles since they lie in a region not of observed fact but of pure phantasy.

connection with the spermatocyte-divisions, are thoroughly decisive in showing the third small chromosome to be an extra *m*-chromosome not distinguishable in any respect from the other two.

2 DISCUSSION

It seems to me that in the individual of *Metapodius* that has here been described nature has performed an experiment which, as far as it goes, is precisely such as we should like to carry out artificially in order to test the hypothesis of the genetic continuity of the chromosomes and the question of their qualitative relations in the maturation-process. The experiment (if we may call it such) consisted in the omission from the typical 22-chromosome diploid groups of the small idiochromosome, and its replacement by one of different type, a third *m*-chromosome. In what way this was effected can only be conjectured; but it seems altogether probable that the anomaly was present in the original fertilized egg, as a result of one preëxisting in one or both the gamete-nuclei.⁵ In any case we may be sure that it arose very early in the ontogeny, at a period prior to the separation of the right and left gonads, since both testes show precisely the same characters.

It is certain that the initial anomaly has persisted unchanged through many generations of cells, and that the alteration in the diploid groups has involved corresponding modifications in the maturation-process. The significant fact is that throughout this process *the chromosome that has been added does not take the place of the one that has been omitted, but behaves according to its own kind.* This is a truly remarkable result when we consider that the number of chromosomes in the diploid groups (22) remains unaltered. These groups still consist of 11 pairs of chromosomes; but one is

⁵ We must assume, in this case, that the sperm-nucleus contained no small idiochromosome and that either this nucleus or the egg-nucleus contained two *m*-chromosomes. The former condition may have resulted from a failure of the idiochromosomes to separate in the second spermatocyte-division (which, as I have shown, may actually occur). The presence of a second *m*-chromosome may be due to a similar cause.

an unnatural or hybrid pair, which consists of non-homologous members—the large idiochromosome and the third *m*-chromosome. The facts show most decisively that these two chromosomes do not play the part of synaptic mates towards each other, but retain each its own characteristic behavior. In synapsis the third *m*-chromosome invariably couples with its own kind to form a triad element while the large idiochromosome remains unpaired. Thus the substitution of one chromosome for another of a different kind has been followed by no regulative process, and a permanently new combination has been produced. The full force of this conclusion first becomes evident when we compare the present case with those in which there is present a single small supernumerary of the type described in my fifth Study. In the diploid groups such a supernumerary is quite indistinguishable from a third *m*-chromosome—as we may see, for instance, upon comparison with figs. 1*k*, 7, *t-y*, photograph 29, of Study V.⁶ In the first spermatocyte-division also, in cases where a small supernumerary lies within the ring of large bivalents (as in photograph 6, fig. 7*i* of my fifth Study) side-views give a picture almost indistinguishable from such a condition as that shown in fig. 3*c*. Such a side view of terminalis No. 1, is given for comparison in fig. 3*d*, the two *m*-chromosomes just separating at the center of the group, and the supernumerary (*s*) just to the left. The resemblance between this figure and fig. 3*c* is so close as to amount almost to identity. It seems incredible that the behavior of the third small chromosome in the ensuing division should not be identical in the two cases; and it should be identical were the history of the individual chromosomes in maturation determined merely by their size or their mechanical relation to the achromatic figure. In point of fact, however, the small supernumerary and the third *m*-chromosome show characteristic differences throughout the whole process. In the growth-period the former appears as a condensed chromosome-nucleolus, usually coupled with the idiochromosome-nucleolus, while the *m*-chromosome remains diffuse and usually free. In the first division the former divides as a univalent (i. e., it is typically uncoupled, though it may be in contact with the idiochromosomes) and is

⁶ Loc. cit.: '09c.

usually outside the ring (as in fig. 2*h*); while the third *m*-chromosome is always coupled with the two others at the center of the ring, and moves to one pole without division. In the second division these relations are almost exactly reversed, the *m*-chromosome dividing equationally as a univalent, while the supernumerary does not divide and is typically coupled with the idiochromosome bivalent near the center of the group. I desire to emphasize the fact that these differences are in no way obscure or difficult to see, but are conspicuously shown in so great a number of cells as to remove all doubt.⁷

⁷This point demands emphasis because of the scepticism expressed by certain writers in regard to the constancy of the chromosomes in respect to number, size and behavior. Conspicuous among these writers is Della Valle ('09) who has brought together a valuable if somewhat uncritical review of the literature, and contributes careful observations of his own upon variations in the chromosome-number in the somatic cells of *Salamandra*. Such scepticism is perhaps not surprising in view of the unlucky contradictions that still exist in the literature even of so favorable and well known a group as the insects. But to ascribe this confusion of the literature to a confusion of the facts—i. e., to an inconstancy so great as to preclude the possibility of attaining exact results—would be, I think, a fatal blunder. The confusion in the literature cannot, of course, be attributed altogether to mistakes of observation or to accidents of technique—though both these must be held to a strict reckoning. I am not aware that anyone has maintained that the relations of the chromosomes form an exception to all other biological phenomena in being absolutely fixed and immutable; and due weight should be given to the numerical variations that have been recorded by Della Valle and many others myself included. The fact remains that it is possible to determine accurately what are the normal or typical relations of the chromosomes, as of other structures, and to establish in many cases their high degree of constancy. The same common sense must be used in the treatment of these relations as in the case of other phenomena that are subject to variation. For example, insects have been seen with seven legs, but it is not for this reason to be doubted that insects have six legs. In like manner, in the ovaries of *Largus cinctus* I have seen as many as three dividing cells that show 13 chromosomes; but I nevertheless do not doubt, after the study of a large number of cases, that the typical number is 12.

The case of *Metapodius* is disposed of by Della Valle in the following easy fashion. "Not constancy but variability in the number of chromosomes is the general rule in all organisms; of which the observations published by him (Wilson) are but a special confirmatory case" (*op. cit.*, p. 161, translation). Better acquaintance with the facts in *Metapodius* would probably render Prof. Della Valle less certain of this; for I am confident that no observer of ordinary competence could confuse such a series of relations as that here displayed with the occasional fluctuations with which we are familiar in many forms, including this very genus.

It seems to me that such facts have the value of actual experimental evidence in support of the hypothesis of the genetic continuity of the chromosomes and that of their qualitative difference. All will admit that the peculiarities of the later generations of cells in this individual of *Metapodius* are inherited from earlier ones. It is the obvious, natural and, I think, inevitable conclusion that the third *m*-chromosome, introduced at an early period, has not lost its identity in the later stages. If its presence is merely owing to a corresponding excess of chromatin, how shall we account for the characteristic peculiarities of behavior that differentiate it so sharply from an ordinary "supernumerary" of corresponding size? To reply that the excess represents a particular kind of chromatin that is re-segregated at each division in the form of a particular chromosome is to grant the most vital assumption in the hypothesis of genetic continuity.

I think that sufficient emphasis has not yet been laid upon the support given to this hypothesis by the variable position of the chromosomes in the diploid groups. I have several times pointed out in this paper and preceding ones, that there is no constancy in the relative position of the spermatogonial chromosomes—as may be seen with particular clearness in case of the *m*-chromosomes of the Coreidæ or the small idiochromosome of the Pentatomidæ or Lygæidæ, or of chromosomes distinguishable by their large size, such as are seen in *Protenor*, *Largus* or *Anasa*. This is certainly not what we should expect were the chromosomes merely "tactic" formations that appear in characteristic array, as a crystal form in a solution, merely because of the specific properties of a single chromatin-substance as such. Two answers might be made to this. It might be said that the chromosomes merely represent the segregation of so many different kinds of chromatin that are mixed together in the resting nucleus.⁵ I am disposed to regard this as a tenable hypothesis; but obviously it grants the most essential part of the continuity hypothesis. Again it might be said that the chromosomes are originally formed always in the same position but lose it by subsequent shiftings in the prophase. It

⁵ Cf. Fick: '05; Wilson: '09c.

would be difficult to disprove this in ordinary cases; but fortunately Boveri's studies on *Ascaris* ('09) have shown beyond all doubt that in this form there is no constancy in the original position of the prophase chromosomes, the only definite order being shown in the close agreement of each pair of daughter-cells. The position of the prophase chromosomes as Boveri shows with great cogency is here a consequence of the position in which they entered the nucleus in the preceding telophases; as the latter position is itself due to causes (which may be quite fortuitous) that determine the position of the preceding metaphase chromosomes.

The facts support no less directly and strongly the conclusion that the chromosomes differ among themselves in a definite way in respect to their behavior, and hence in respect to their functional significance. The differences seen in the maturation-process have thus far taught us nothing whatever in regard to the individual physiological meaning of the chromosomes, in heredity or otherwise, and they are not to be compared in value with the results of direct experiment, such as those carried out by Boveri ('07) in dispermic sea-urchin eggs. It is nevertheless of great interest that the results from these different sources should be in harmony. In my preceding paper I have called attention especially to the significance of the couplings of the chromosomes, pointing out that these certainly do not depend upon the size of the chromosomes (though those which couple in synapsis are in fact equal members of a pair save in certain special cases) nor can they, apparently, depend upon the achromatic mechanism. The various combinations in *Metapodium* seem to arise simply by the addition or subtraction of certain chromosomes without alteration of the achromatic elements; yet in the resulting new combinations the chromosomes still behave each according to its kind, and (as previously indicated) irrespective of their size. We seem thus driven to accept the view that the chromosomes are physico-chemically different, with all the consequences which such a view may involve.

The cogency of the evidence in favor of the qualitative differences of the chromosomes brought forward in Boveri's masterly work must be generally recognized, as has recently been admitted even by Driesch ('09) who formerly endeavored to find a different

explanation of the facts. It will be evident to readers of my former papers that I am fully prepared to accept Boveri's conclusions; but there is one very important fact, finally established by the present paper, that must be clearly recognized. If we assume that different factors of heredity are in some sense unequally distributed among the chromosomes, we need feel no surprise that the duplication of one or more of the ordinary chromosomes should produce no perceptible qualitative effect upon development. But it is very surprising that no visible effect should be produced by the removal of a particular chromosome that has no duplicate to take its place. In preceding papers I have called attention to the singular fact that Montgomery's material of *M. terminalis* differs consistently from my own in the lack of the small idiochromosome or "Y-element" (see Wilson '09a, for this term); but the possibility of two distinct species or races having been confused could not be absolutely excluded. In the present case, however, no doubt can exist, since the two original specimens of *M. femoratus* from Miami, Florida, are in my cabinet, and in perfect condition for identification. One of these, as already stated, contains both the small idiochromosome and an additional supernumerary, while both are lacking in the other individual; yet the two individuals seem to be otherwise in every essential respect identical. All doubt is thus removed that the small idiochromosome or Y-element, which forms the synaptic mate of the accessory chromosome or X-element, may be present in some individuals and absent in others of the same species, without the appearance of any corresponding differences in the sexual or other characters as far as shown in the external morphology of the animal.⁹ This chromosome, as shown

⁹ It will be seen from this how readily discrepancies regarding the number of chromosomes might arise between different observers working on the same species. It might seem that we have here a simple and plausible explanation of the contradictions that have arisen in the case of *Anasa tristis*; for we might assume that the diploid number is 21 in some individuals of this species and in others 22; and a similar explanation has in fact already been adopted by more than one recent writer (cf. Della Valle: '09, Buchner: '09b).

I am not myself able to take this view of this particular case for several reasons. In the first place, if there be individuals of this species that have 22 spermatogonial chromosomes, as maintained by Foot and Strobell ('07) we should expect to see

by the work of Stevens and myself, is widely distributed among the insects (Hemiptera, Coleoptera, Diptera) and is strictly confined to the male line (except when supernumeraries are present). In species having an odd or accessory chromosome the Y-element (small idiochromosome) is wanting, and I have urged this fact as showing that this latter chromosome cannot play any essential rôle in sex-production¹⁰ or in the transmission of the secondary sexual characters, as Castle ('09) ingeniously suggests. What I desire here to point out is that by parity of reasoning we should also conclude that this chromosome is devoid of any special significance in heredity of any kind, at least as far as the visible external charac-

12 instead of 11 separate chromosomes in the first spermatocyte division, as we do in the 22-chromosome individuals of *Metapodius* (Wilson: '09c). Throughout the Hemiptera, indeed, when the accessory chromosome (or its homologue, the large idiochromosome) is accompanied by a synaptic mate or Y-element, the two are separate in the first division, which accordingly shows one more than the reduced or haploid number—i.e., $\frac{n}{2}+1$. The photographs of Foot and Strobell show, however, 11 chromosomes in this division (the two *m*-chromosomes being of course counted as one, like the other bivalents), as they should if the spermatogonial number be 21.

Still, this might be a case like that of *Syromastes*, where no Y-element is present, but the accessory is itself double—though such a parallel would hardly help the case, since in no form is the failure of the accessory to divide in one division more indubitably shown than in *Syromastes*, while Foot and Strobell are persuaded that it does divide in *Anasa*. But, secondly, my own extended additional observation, like the studies of Lefevre and McGill ('08), still continues to give but one result as before. The living animals (from the same locality as the material of Foot and Strobell) have been kept by hundreds in the greenhouse for months at a time in successive years, and have been regularly employed for class work in cytology and for experimental purposes, in the course of which large numbers of additional sections and smears have been prepared and examined. Others as well as myself have carefully searched among these preparations for cases showing more than 21 spermatogonial chromosomes, without success apart from the double or multiple groups that occasionally appear. The same relation continually recurs, namely 21 spermatogonial chromosomes of which three are larger than the others, while in the dividing ovarian cells the number 22 appears with equal constancy. That not even one case of 22 spermatogonial chromosomes has thus far been found is indeed surprising; for plus variations in the diploid groups are known to occur in some species of Hemiptera, and I have myself described such cases (*e. g.*, Wilson: '09c).

These and other reasons lead me to believe that the conclusions of Foot and Strobell were based on the observation either of very rare fluctuations in the normal diploid number or of accidental products of the technique.

¹⁰ Loc. cit.: '06, '09a, '09d.

ters and the more obvious internal features are concerned. Taken by itself this case may seem to form a legitimate piece of evidence against Boveri's theory. It cannot, however, be taken alone, but must be viewed from the more general standpoint given by the evidence as a whole. We are still too ignorant of the significance of the "sex-chromosomes" to form an adequate opinion as to the meaning of the Y-element. It may be in this case (as I earlier suggested) a degenerating element, or may represent an excess of a chromatin that is duplicated elsewhere in the chromosome-group; but these and other speculative possibilities that suggest themselves may well await the outcome of further study.

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REPRODUCTION AND PARASITISM IN THE UNIONIDÆ

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THIRTY-NINE FIGURES

FIVE PLATES

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INTRODUCTION

The threatened extinction in the Mississippi River and its more important tributaries of those species of the Unionidæ whose shells have been taken in enormous numbers during the past fifteen years for manufacture of pearl buttons has led the United States Bureau of Fisheries to undertake an extensive investigation to determine the possibility of artificial propagation of the commercial species, and to devise such means as may be practicable of restocking depleted waters which present favorable conditions for their growth. The general direction of the investigations has been placed in the hands of the writers, who for the past three years have devoted as much time as their other duties

have allowed to the work, in certain phases of which, however, many others have collaborated.

The plan of the work has embraced, besides a thorough investigation of artificial propagation, a detailed study of the life history and ecology of the Unionidæ, emphasizing especially the geographical distribution of the group throughout the Mississippi Valley, breeding seasons and habits, physical conditions of the waters in which the different species thrive, food enemies, diseases, rate of growth, and the behavior of glochidia and fishes, as parasites and hosts, respectively. Although much yet remains to be done, results of importance have been obtained, and the entire feasibility of artificial propagation clearly demonstrated.

The writers' personal attention has been largely directed to a study of the conditions of reproduction in the group and to the parasitism of the larvæ from the point of view of their relation to artificial infection of fishes with the glochidia, while such phases of the investigation as geographical distribution, enemies and diseases and general habits have been carried on by others.

It is through the kindness of the Commissioner of Fisheries, the Honorable George M. Bowers, that we have the privilege of publishing, in advance of our detailed report, certain results which bear upon the study of reproduction and parasitism and which are briefly presented in this paper. It also gives us pleasure to express here our appreciation of the enthusiastic support which has been given us by Dr. Barton W. Evermann, Chief of the Division of Scientific Inquiry of the Bureau of Fisheries, whose unflinching interest and faith in the investigations since their beginning have been largely responsible for such success as has been attained.

As has long been known, the Unionidæ carry their young in the gills, which function as brood-pouches until the completion of the embryonic development. At the close of this period, the larva or so-called glochidium is fully formed and escapes from the egg-membrane while still within the gill. In some species the discharge of the glochidia takes place at once, although in others

they remain without further change in the brood-pouches for several months before being set free in the water.

The glochidium, long thought to be a parasite infesting the gills and known as *Glochidium parasiticum*, was proved by Carus in 1832 to be the larva of the mussel itself, although Leeuwenhoek many years before had correctly interpreted its relation. In 1866 Leydig made the important discovery that the glochidium, after leaving the parent, completes its development as a parasite on fishes. .

Since an adequate review of the literature relating to the embryonic development and the parasitism of the larva has quite recently been published by Harms ('09), an historical account of the subject may be omitted here and reference had to his interesting and valuable paper.

THE MARSUPIUM

The term *marsupium* has been generally used to indicate those portions of the mussel's gills into which the eggs are received from the suprabranchial chambers after ovulation and which serve as brood-pouches for the retention and nurture of embryos and glochidia until the discharge of the latter. As no better name seems to be available, we shall employ it in this paper. Since the extent to which the gills are specialized for this purpose varies in different groups of the Unionidæ, Simpson ('00), in his Synopsis of the Naiades, has made use of the marsupium as the chief diagnostic character on which his classification is based. Those groups in which the marsupium comprises the outer or all four gills he designates as the exobranchiæ, while those in which the inner gills alone receive the eggs are distinguished as endobranchiæ. All of the European and North American species belong to the former group, while the latter contains forms that are found chiefly in Asia, Australia, Africa, Central America, and South America. As our observations have been confined to the exobranchiæ, reference will be made only to this group, the following subdivisions of which are recognized by Simpson, each distinguished by special marsupial characters.

Tetragenæ. Marsupium occupying all four gills.

Homogenæ. Marsupium occupying entire outer gills.

Heterogenæ. Marsupium occupying only posterior end of outer gills.

Mesogenæ. Marsupium occupying a specialized portion in the middle region of outer gills.

Ptychogenæ. Marsupium occupying entire outer gills which are thrown into a series of peculiar folds.

Eschatigenæ. Marsupium occupying the lower border only of outer gills.

Diagenæ. Marsupium occupying entire outer gills, but differing from that of the *Homogenæ* in that the egg-masses lie transversely in the gills.

Simpson has established another group, the *Diagenæ*, for the genus *Tritogonia* but since its marsupium is constituted by all four gills it should at least be included in the *Tetragenæ*, if not in the genus *Quadrula*, as Ortmann suggests ('09).

It will be seen from the above classification that three general conditions exist, namely one in which the marsupial adaptation, involves all four gills; one in which the entire outer gills only are utilized; and, lastly, one in which some differentiated portion of the outer gills constitutes the marsupial region.

For a complete list of the genera occurring in each of Simpson's groups, reference may be had to his *Synopsis* (*l. c.*, pp. 514-515), but since some of the types have not been adequately described and figured, we have inserted here an account of the several marsupial modifications which have come under our own observation, together with a list of the species in which we have found them.

Tetragenæ. The marsupium in these forms comprises all four gills which when gravid are distended and present a smooth pad-like appearance. It is the condition found in the genera *Quadrula* and *Tritogonia*. We have encountered it in *Tritogonia tuberculata* Branes, and in the following nine species of *Quadrula*: *ebena* Lea, *heros* Say, *lachrymosa* Lea, *metanevra* Rafinesque, *obliqua* Lamarck, *plicata* Say, *pustulosa* Lea, *trigona* Lea, and *undulata* Barnes. Fig. 4, which is drawn from a gravid female of

Quadrula ebena, illustrates the typical appearance of the marsupium in this group, although the gills, as they appear in the figure, are not as fully distended as is frequently the case. In many species of the genus the eggs and embryos are brilliantly colored, red or pink, and, when the marsupium is charged, the color shows through the transparent walls of the gills, which present a striking appearance on removing the shell.

There has been a certain amount of discussion among the conchologists as to whether, or not, the functioning of all four gills as a marsupium is a constant character in *Quadrula*, and observations have been to a certain extent conflicting. Since Simpson has made use of this feature in characterizing the group *Tetragenæ*, some importance has been attached to the apparent discrepancy in observations. Ortmann (*l. c.*, p. 101), for example, states that in a dozen specimens of *Q. coccinea* only the outer gills were charged with embryos. "This would remove" he says "this species from the genus *Quadrula*, and would place it with *Pleurobema*. Baker ('98, p. 80), gives a description of the soft parts, and says, "four gills used as marsupium," but this may not be founded upon personal observations, but may have been inferred from the systematic position of the species."

While examining mussels on the upper Mississippi River in the summer of 1908, we observed a peculiarity in behavior in all of the species of *Quadrula* collected which probably accounts for the conflicting descriptions of the marsupium in this genus, and also for the fact that in some species gravid females have never been observed at all. Every species of *Quadrula* that came into our hands exhibited to a greater or less degree the habit of aborting embryos and glochidia, when taken out of the river, and if they were not opened and examined at once upon capture, they were generally found shortly afterwards to be either partially or entirely empty. Some individuals discharged the contents of their gills more readily and completely than others, the abortion involving either all four gills, or only the inner or outer ones, or, again, only a portion merely of one or more gills. In the pre-glochidial stages, when the embryos are conglutinated, the entire masses were discharged, while individuals were frequently seen in the act of abort-

ing their embryos or glochidia which were often expelled with considerable force through the exhalant siphon. This behavior was so characteristic of the genus that, in order to make a correct determination of the condition of the marsupium, it was necessary to open *Quadrulas* immediately after taking them from the water. When this was done, all four gills were invariably found to be charged on opening females which contained embryos in pre-glochidial stages, that is, at any time before normal spawning had occurred. The habit of aborting embryos, when disturbed has, been observed by us in but one species not belonging to *Quadrula*, namely, in *Unio complanatus*, which has been repeatedly seen in the act of discharging the contents of the marsupium shortly after being placed in the aquaria of the laboratory at Woods Hole, Mass. In all likelihood it occurs in other species of *Unio*, and it has been referred to by Schierholz ('88) and Latter ('91) as taking place in *Anodonta*. It is probably due, as Schierholz suggests, to imperfect aëration of the water, and if this is true, one would not expect to find it in those mussels which have a differentiated marsupium, like the *Heterogenæ*, since in such forms the respiratory and marsupial functions of the gills are not so intimately associated.

Homogenæ. The condition in which only the outer gills are utilized as a marsupium is present in sixteen genera according to Simpson. We have verified its occurrence in *Alasmidonta truncata* Wright; *Anodonta cataracta* Say, *grandis* Say, *implicata* Say; *Arcidens confragosus* Say; *Pleurobema æsopus* Green; *Symphynota complanata* Barnes, *costata* Rafinesque; and in *Unio complanatus* Dillwyn and *gibbosus* Barnes. The outer gills when filled with embryos or glochidia may be greatly distended beyond their normal dimensions, and in this condition are enormously swollen pad-like structures filling a large portion of the mantle chamber. Fig. 6 represents the marsupium of *Symphynota complanata* which may be taken as typical of the class.

Heterogenæ. In this group the marsupium occupies only the posterior portion of each outer gill, varying in extent from about one-third to two-thirds of the entire length of the latter. It is permanently differentiated, being sharply marked off from the res-

piratory portion either by a deep notch or a distinct fold and projecting further down into the mantle chamber, as it is much deeper dorso-ventrally than the non-modified anterior end of the gill. Unlike that of the Tetragenæ and Homogenæ, the marsupium in the Hertogenæ is readily recognized when empty, for, in addition to its permanent demarcation, its walls are thinner and more membranous in appearance than those of the respiratory portion, and after discharge of the glochidia it is seen as a flabby collapsed pouch. The inflation of the posterior end of the valves which is characteristic of the females in the Heterogenæ is associated with this type of marsupium, as it allows of increased space inside the shell for the accommodation of the gravid gill. Owing to this enlargement of the shell, which is absent in the males, the sexes are externally distinguishable in the Heterogenæ.

The typical condition is seen in the genus, *Lampsilis*, as shown in fig. 2, which is drawn from a gravid individual of *L. subrostratus*. The extent of the marsupial modification, however, varies in different genera and even in different species of the same genus, occupying, as already stated from about one-third to two-thirds of the length of the entire outer gill. Fig. 5 illustrates the marsupium in *L. rectus*, in which it forms the posterior third of the greatly elongated outer gill and is sharply marked off from the respiratory region by a deep notch. The last two figures indicate the two extremes of the differentiation in this group. Simpson has included fourteen genera in the Heterogenæ, only three of which, however, have come under our observation, namely *Lampsilis*, *Obovaria*, and *Plagiola*. We have recorded this type of marsupium in *Lampsilis* (*Proptera*) *alatus* Say, *anodontoides* Lea, *gracilis* Barnes, *higginsii* Lea, *lævissimus* Lea, *ligamentinus* Lamarck, *luteolus* Lamarck, *rectus* Lamarck, *subrostratus* Say, and *ventricosus* Barnes; in *Obovaria* *ellipsis* Lea; and in *Plagiola* *elegans* Lea and *securis* Lea.

Mesogenæ. This group is so designated by Simpson to include two genera, *Cyprogenia* and *Obliquaria*, in which a variable number of enlarged water-tubes in the middle region of the outer gill are specialized as the marsupium, the portion of the gill both in front and behind retaining its ordinary respiratory character. We

have encountered the condition in one species only, *Obliquaria reflexa* Rafinesque, which is represented in fig. 3. Here the distended tubes project far down below the lower border of the rest of the gill, and when gravid appear greatly swollen. The marsupial region is permanently differentiated and is easily recognized when empty. The number of tubes comprising the marsupium varies in different individuals of the species from two to eight and during the breeding season each tube is filled by a solid cord of a stiff glutinous consistency in which the embryos or glochidia are embedded.

Ptychogenæ. This group contains a single genus, *Ptychobranchus*. The marsupium occupies the entire outer gill which is thrown into a series of folds, each water-tube ending below in a swollen bulb-like enlargement, as seen in fig. 1, which is drawn from *P. phaseolus* Hildreth. Simpson (*l. c.*, p. 612) states that the number of folds varies from six to twenty. In the specimen figured there are seventeen.

Eschatigenæ. Simpson has established this group for the genus *Dromus* in which the marsupium occupies the lower border only of the entire outer gill, the ventral ends of the water-tubes being slightly distended and rounded. The condition, which has not been seen by us, would seem to differ from the marsupium of *Ptychobranchus* chiefly in the absence of the folds.

Diagenæ. Here the outer gills throughout their entire length are utilized as a marsupium, which in external appearance is quite similar to that of the *Homogenæ*. The *Diagenæ*, however, differ from other *Unionidæ* in that the embryos and glochidia are contained in peculiar cylindrical sacs which lie *transversely* in the gills, whereas in every other case the egg-masses are placed vertically in the gills. The group contains one genus, *Strophitus*. We have observed the gravid marsupium in *S. edentulus* Say, although we have not had an opportunity of determining whether the unusual position of the egg-masses is correlated with some peculiarity in the structure of the water-tubes, or not.

It is probable as Simpson concludes (*l. c.*), that the oldest type of marsupium phylogenetically is that occurring in the *Endobranchiæ* in which the inner gills only are used as brood-chambers. It is a slight transition from this condition to that existing in the

Tetragenæ with all four gills functioning for this purpose. Basing his supposition largely upon shell characters and facts of geographical distribution, he further concludes that the Homogenæ marked the next step in marsupial differentiation, while the Heterogenæ and all other groups in which a portion of the outer gills only is structurally modified for receiving the eggs are the latest product of the evolution of the Unionidæ. That this series correctly represents the phylogenetic sequence in the appearance of the marsupial modifications would seem to be borne out by the structural conditions existing in the several types, so far as we have examined them.

BREEDING SEASONS

In connection with our study of artificial propagation of freshwater mussels, we have found it necessary to collect data bearing upon the breeding seasons of a fairly wide range of species, since the records of previous observers, for North American Unionidæ at least, have been insufficient to enable us to determine the full extent of the seasons, especially in the case of some of the more important commercial species. Although our observations have been largely confined to species occurring in the upper Mississippi Valley and have been concerned primarily with species of commercial value, we have continuous records throughout the entire year for a number of important genera, and in every case the exact stage of development of the embryos has been determined by microscopic examination. Many thousands of such observations have been made, so that we are now in possession of detailed information dealing with the duration and progress of the periods of gravidity obtaining in over a dozen genera of the Unionidæ.

We have confirmed the conclusion first reached by Sterki ('95) that the North American Unionidæ, with respect to their breeding seasons, fall into two classes, the so-called "summer breeders" and "winter breeders." The latter designation, however, is not strictly appropriate, for in the species which belong to this group, the eggs are fertilized during the latter part of the summer, usually in August, and the glochidia, which are carried in a fully developed

condition in the marsupium throughout the winter, are not discharged until the following spring and summer. In fact, in some cases the close of one breeding period may overlap on the beginning of the next, as one may still find in late July a few straggling females gravid with glochidia formed in the previous autumn, while in other individuals of the species at the same time and in the same locality the eggs are passing into the gills for the next season. This seems to be true of several species of *Lampsilis*. We have encountered it in *ligamentinus*, Conner ('09) records it for *radiatus* and *nasutus*, while Ortmann ('09) states that his observations make it probable for *ventricosus* and *luteolus*. Yet, as Ortmann observes, it is generally true that an interval exists between the close of one period and the beginning of the next. This interval, however, varies in length with different species, in some extending from late spring until August, whereas in others it is of much shorter duration. It is also to be noted that the discharge of glochidia does not take place in all of the individuals of a species at the same time, but on the contrary spawning may extend over a considerable period throughout the spring and early summer (*cf.* Ortmann, *l.c.*).

In the case of the summer breeders, the eggs are fertilized during late spring and summer, and spawning as a rule is over by the end of August. In the species belonging to this group of which we have the completest records, ovulation begins in May and early June and may continue throughout July, while after the end of August gravid females have not been found.

In view of the above facts, it would seem to better accord with the actual conditions to separate the species with respect to the length of time that the glochidia remain in the marsupium, designating them as those that have a "short period" and those with a "long period" of gravidity, rather than to distinguish them as "summer breeders" and "winter breeders," respectively, for with respect to the latter neither ovulation nor discharge of the glochidia takes place in winter.

The breeding season is undoubtedly a generic character, for, so far as our observations have gone, all of the species belonging to a given genus have essentially the same period of gravidity.

Although our records in detail for each species will be published in our forthcoming report to the Bureau of Fisheries, the following lists show the distribution, with respect to the long and the short breeding seasons, of the genera which have come under our observation.

Long period of gravidity.—*Alasmidonta truncata*; *Anodonta cataracta*, *grandis*, *implicata*; *Arcidens confragosus*; *Lampsilis* (*Proptera*) *alatus*, *anodontoides*, *gracilis*, *higginsii*, *lævissimus*, *ligamentinus*, *luteolus*, *rectus*, *subrostratus* *ventricosus*; *Obovaria ellipsis*; *Plagiola elegans*, *securis*; *Strophitus edentulus*; *Symphynota complanata*, *costata*.

Short period of gravidity.—*Obliquaria reflexa*; *Pleurobema æsopus*; *Quadrula evena*, *heros*, *lachrymosa*, *metanevra*, *obliqua*, *plicata*, *pustulosa*, *trigona*, *undulata*; *Tritogonia tuberculata*; *Unio complanatus*, *gibbosus*.

Ortmann ('09) has recently published some observations on the breeding seasons of the Unionidæ of Pennsylvania, supplemented by data from Lea and Sterki. and although he merely records a species as gravid or not in a given month, his results in all essential points agree closely with ours. He includes among "winter breeders" several genera which we have not had under observation, namely, *Truncilla*, *Micromya*, *Cyprogenia*, *Ptychobranhus* and *Anodontoides*, while *Arcidens* and *Obliquaria*, which we have recorded, do not appear in his lists of "winter breeders" and "summer breeders," respectively. Although in many cases we have had the same species under observation, his records include a number that are absent from ours, while our list supplements his especially by the addition of several species of *Quadrula*, for which data have previously been quite meagre.

By examining the two sets of observations, it will be found that, whereas the *Homogenæ* and *Mesogenæ* are represented in both groups, some genera in each having the long period and others the short period of gravidity, the *Heterogenæ*, *Ptychogenæ*, and *Diagenæ* are so-called "winter breeders" exclusively, while the *Tetragenæ* breed only in the summer.

The breeding seasons, as defined above, are based upon data col-

lected in the middle and northern sections of the United States, and in the absence of adequate records from higher and lower latitudes, it is impossible to say to what extent a colder or a warmer climate might affect the period of gravidity. That it would have some influence can hardly be doubted, although a distinction between a long and a short season will probably be found to hold true in general.

The same difference has been repeatedly stated to occur among European Unionidæ. According to Harms ('09, p. 332), for example, in *Unio* the breeding season begins early in March, or, if the weather is cold, not until the end of May. In *Anodonta*, which carries the larvæ over the winter, the eggs are fertilized about the middle of August, all of the individuals entering upon the breeding season at nearly the same time, while by the middle of October almost all the females are gravid with glochidia. *Margaritana* breeds in July and August, according to Harm's observations, and during that time produces two successive broods, from sixteen days to four weeks, according to the temperature, being required for the development of each. Although we have not determined it beyond all doubt, our records strongly indicate that the species of *Quadrula*, and possibly some other summer breeders, also spawn twice during the same seasons, first in June or July and again in July or August.

CONGLUTINATION OF THE EMBRYOS

After extrusion of the eggs from the genital aperture, they are passed along by ciliary action to the cloaca, and thence by suction according to Latter ('91, p. 53), are drawn back into the supra-branchial chambers. Here they are fertilized by spermatozoa brought in with the incoming current of water, and then pass into the water-tubes of the gills, eventually filling up those portions which function as the marsupium.

As the eggs settle down into the gills, they come into contact with the secretion formed by the glandular epithelium lining the tubes, which in many cases is of a glutinous nature, although its consistency varies in different species. It is, however, usually

quite tenacious. In a short time after entering the marsupium, the eggs become conglutinated into masses which, as the mucilaginous matrix stiffens, are molded into the exact shape of the cavity of the water-tube, of which each mass forms a cast. The masses are of course separated from each other by the intervening interlamellar junctions of the gills.

Since it is a matter of convenience to have a word to apply to these compact masses, in which the eggs or embryos are held together, whether they be plate-like, wedge-shaped, cylindrical or of some other form, we shall employ the term *conglutinate* in referring to them, as being perhaps the best one available for the purpose. They have generally been called "ovisacs" by the American systematists, but this is of course misleading, as they are not sacs in any sense, but merely masses of eggs adhering to one another by means of a cement.

The conglutinates vary greatly in size and shape in conformity with the special conditions of the marsupium existing in the different types. The commonest form is that of a flat oval plate, slightly blunter and thicker above and more pointed and thinner below. Such plates, differing, however, in size and thickness, are characteristic of *Lampsilis*, *Quadrula*, *Unio*, and many other genera. In fig. 27 two of the conglutinates of *Lampsilis ligamentinus* are represented, one from the flat side, the other on edge. In those genera in which the size and form of the water-tubes of the marsupium depart more widely from the usual condition, the conglutinates are similarly modified. In *Obliquaria reflexa*, for example, in which the marsupium consists of several elongated and distended water-tubes in the middle region of the outer gill, the conglutinates are unusually large, being slightly curved cylindrical masses of nearly uniform diameter and generally blunt at each end. Three of them are shown in fig. 26. The one on the right was taken from the most posterior water-tube of the marsupium which is not as long as the rest and its conglutinate is correspondingly shorter. The relation will be understood by reference to the figure of the marsupium in this species (fig. 3).

In those species, *e. g.*, *Unio complanatus*, in which the antero-posterior diameter of the water-tube is scarcely greater than that

of the egg, the conglomerates are very thin and as a rule are composed of but a single layer of eggs, while in others, as in the species of *Lampsilis*, the plates are much thicker, especially above, and in horizontal section show several layers. In the genus *Quadrula* the thickness is intermediate.

The amount of the matrix in the conglomerates also varies considerably in different species. In *Unio complanatus* and *Obliquaria reflexa*, the egg membranes, which seem to be of an adhesive nature, are closely pressed together in the mass on all sides, as shown in fig. 28, which is a detail drawn from one of the conglomerates of fig. 26. The glochidia are seen with the valves open but still contained within the membranes, which are closely adhering. In cases like this, it is difficult to determine whether there is any cement substances at all between the embryos, which may possibly be held together solely by means of the adhesive surfaces of the membranes. In many other cases, however, the matrix is quite evident, and the embryos, which are not so closely appressed, are simply embedded more or less loosely in a glutinous binding substance. Such is the condition in *Lampsilis* and is illustrated in fig. 24, which is a portion of one of the conglomerates of fig. 27 seen under higher magnification.

In still other species, the eggs cannot be said to form conglomerates at all, as they are merely suspended in a slimy mucus which never hardens sufficiently to enable the mass to maintain a definite form when removed from the gill. This condition is most noticeable in *Alasmidonta truncata*.

When the glochidia are fully formed, the cement, which throughout the embryonic development has held the embryos together, dissolves away, and the larvæ are discharged at the time of spawning in slimy strings of mucus which is secreted by the lining epithelium of the marsupium.

HISTOLOGICAL STRUCTURE OF THE MARSUPIUM

Figs. 30 and 31 are horizontal sections of water-tubes of the marsupium of *Quadrula ebena*, and *Lampsilis* (*Proptera*) *alatus* respectively, and, as they are drawn under the same magnification, a comparison of the size of the tubes in the two cases may be readily

made. Both contain embryos in an early cleavage stage, only a few of which, however, are represented. The actual sections show the spaces closely packed with embryos. In fig. 31, which may be taken as typical of the condition existing in the *Heterogenæ*, the tubes are not only enormously enlarged, but their walls are relatively thinner, and the whole marsupium has a more membranous appearance than in the *Quadrula* and *Unio* types.

The glandular epithelium is found chiefly on the surfaces of the interlamellar junctions, and in some species is much more highly developed and more extensive than in others, often causing the surface to be thrown into irregular folds and ridges. This is very pronounced in *Unio* and *Quadrula*. When highly magnified, as in fig. 32, the epithelium, resting upon a base of connective tissue and smooth muscle fibers, is seen to be composed of greatly swollen cells, whose vacuoles are filled with a clear mucus-like colorless fluid. Scattered among the large cells and seemingly often lying within the vacuoles, are seen several smaller and darker nuclei which are undoubtedly the nuclei of leucocytes. In fact, there can be little doubt that the epithelium becomes infiltrated with wandering blood cells from the underlying sinuses in the interlamellar junctions. Many indications are present that seem to show that these cells actually wander through the epithelium into the cavities of the water-tubes but what their ultimate fate is, if this be the case, we are as yet unable to say. Possibly they may be ingested by the glochidia and used as food.

STRATIFICATION OF UNFERTILIZED EGGS

It not infrequently happens that eggs pass into the marsupium without being fertilized, and remain there throughout the period of embryonic development, as one may find them in the same gill with fully formed glochidia. In some individuals we have found every egg in the marsupium in this condition. Such eggs have been encountered, however, only in summer breeding species and they seem to be especially common in the genus *Quadrula*, nearly every gravid female of which has been found to contain at least some unfertilized eggs. After remaining in the marsupium for

a time, such eggs generally become swollen and stratified into three distinct layers, a heavier, often pigmented, mass at one pole, a clear or hyaline intermediate zone, and a small granular cap at the lighter pole. As the eggs lie in a constant position in the gills, which are placed vertically in the normal position of the animal it cannot be doubted that the stratification is produced by gravity. It has not yet been determined whether the substances which occur in these layers are the same as would be separated out by centrifuging, or not, but this is not at all unlikely. As many of the species of mussels in which we have seen this condition, for example *Quadrula ebena*, *Q. trigona*, and *Pleurobema æsopus*, have brightly colored red or pink eggs, the stratification is quite striking, the pigments being always at the heavier pole, as it is invariably directed towards the lower border of the gill.

STRUCTURE OF THE GLOCHIDIUM

As has long been known, two well marked types of glochidia are found in the Unionidæ, one provided with stout hooks on the ventral margin of the valves, the other in quite different shape and entirely hookless. The first is characteristically parasitic on the fins and the other external parts from which scales are absent, the second upon the gill-filaments. The occurrence of the two types in the genera which we have examined is shown in the following list:

HOOKLESS GLOCHIDIA

Lampsilis
Obliquaria
Obovaria
Plagiola
Pleurobema
Quadrula
Tritogonia
Unio

HOOKEI

Anodonta
Strophitus
Symphynota

In addition to these, there is the peculiar larva of *Lampsilis* (*Proptera*) *alatus*, which has been called "axe-head" glochidium (fig. 25). This possesses hooks which are not homologous with those of the anodonta type and is to be regarded as more nearly related to the hookless forms, an interpretation which is

borne out by the fact that the "axe-head" can be readily imagined as a modification of the glochidial outline seen in some species of *Lampsilis*, which like *subrostratus* show an approach to a rectangular outline. Its four hooks are so arranged that those of one valve pass inside the opposite ones, thus bringing the ventral margins close together and giving a very firm hold upon the host's tissue. In other respects, it does not show marked differences from the hookless type and the few experiments we have made with it indicate its attachment to the gills rather than the fins.

The detailed structure of each type of glochidium, as found in *Anodonta* and *Unio*, respectively, has been quite well known for many years and recently Harms ('09) has studied them with even more care. We have nothing to add to this except that we have observed concerning the larval thread (formerly erroneously termed the byssus), which we will mention, since the current accounts in zoological text-books and literature lead one to believe that this structure is a conspicuous feature of all glochidia, which is not the case, although it does happen to be present in the European forms which have been most studied. We find the larval thread present in the species of *Unio* and *Anodonta* which we have been able to examine with care and the thread is probably a characteristic of these genera. We have never seen any sign of such a structure in the ripe glochidia of the other genera above listed as possessing hookless glochidia, nor in the hooked forms of the genus *Symphynota*. Lillie ('95, p. 52) considers the thread a condensed excretory product, which, accepting the account of Schierholz ('88), he thinks has become an organ which is of use in bringing the glochidium in contact with the fish. This latter function is the one commonly ascribed to the thread. While we have not studied the pre-glochidial stages in the development of those species which show no thread gland in the mature glochidium (although it is important that this should be done with a view to determining whether any homologue of the thread gland is present at any time), we do not know, that repeated examination of glochidia, either ripe or well along in their development, in several species of *Lampsilis*, particularly *ligamentinus*, *rectus*, *anodontoides*, *ventricosus*, *luteolus* and *subrostratus*, and

to a lesser extent in species of the other genera mentioned, has never shown any trace of the thread which is so conspicuous a feature of the glochidium of *Unio complanatus*. We have also examined the glochidia of *Symphynota complanata* many times with the same negative results and a smaller number of observations confirm this for *S. costata*. Since many species thus have no thread in any way functional for attachment to the fish, the question arises whether the thread when present has as important a function in this respect as has been supposed. Our observations upon the glochidia of *Anodonta cataracta* confirm the descriptions of Schierholz ('88) and others who have studied the European species of *Anodonta*, as to the tangling of the glochidia into masses by means of their extruded threads, and in this genus the threads do seem effective in drawing other glochidia into contact with the fish when a single one has become attached. This is not, however, effective for the greater part of the period during which the glochidium may remain alive upon the bottom, for the threads are dissolved within a day or two and then the glochidia become entirely free from one another. When taken from the parent gill, the glochidia of *Symphynota* are entangled in a ropy mucus and this acts in a manner similar to the threads of *Anodonta*, but it is usually dissolved after a few hours in the water. In the ripe glochidium of *U. complanatus*, the threads are extruded when the glochidia are removed from the parent and placed in water. When this extrusion has taken place, the glochidia and broken egg-membranes become united into globular masses from which it is difficult to separate individual specimens, and from observing such glochidia in contact with fish, we are forced to conclude that they are not so likely to become attached to the gills or fins as they are when separated by the disintegration of the threads of mucus. The glochidia of *Lampsilis*, which when fully ripe, at once spread out into masses of entirely unconnected individuals, appear much better able to attach to the gills of fishes. Accordingly, we would consider the thread as something to be gotten rid of rather than an organ of great importance in the attachment, and this is in agreement with Lillie's interpretation of the thread as an excretory product.

There is considerable diversity in size among glochidia even from the same genus, as represented by the series of text-figures (fig. 1, A-N), all drawn to the same scale, the most striking being the difference between the two species of *Plagiola* (G and H) and between *Lampsilis rectus* and *gracilis* (K and L). Harms ('09) who has studied the exceedingly minute glochidia of *Margaritana margaritifera*, finds that they are exclusively gill parasites

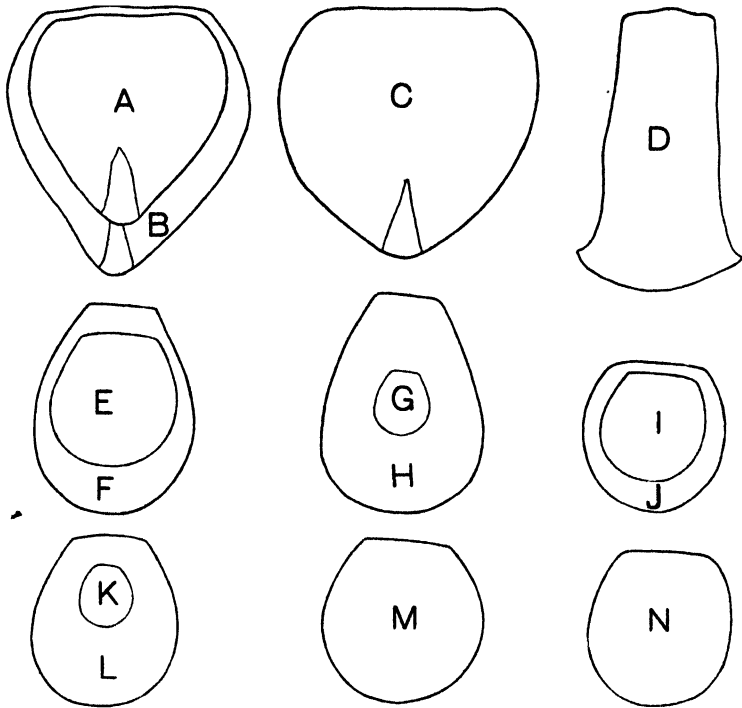


FIG. 1. Figures showing relative sizes and shapes of the shells of a series of glochidia, belonging to the following species: A, *Symphynota complanata*, 0.30×0.29 mm.; B, *S. costata*, 0.39×0.35 mm.; C, *Anodonta cataracta*, 0.36×0.37 mm.; D, *Lampsilis* (Proptera) *alatus*, 0.41×0.23 mm.; E *Quadrula meta nerva*, 0.19×0.18 mm.; F. *Q. pustulosa*, 0.32×0.23 mm.; G, *Plagiola elegans*, 0.09×0.075 mm.; H, *P. securis*, 0.31×0.23 mm.; I, *Quadrula ebena*, 0.15×0.14 mm.; J, *Q. plicata*, 0.21×0.20 mm.; K, *Lampsilis gracilis*, 0.085×0.075 mm.; L, *L. rectus*, 0.24×0.20 mm.; M. *Obliquaria reflexa*, 0.23×0.225 mm.; N, *Unio gibbosus*, 0.22×0.19 mm.

because their small size makes attachment elsewhere impossible. The type of glochidium is constant for the genus, so far as our observations go, and in some cases the shape is also characteristic, as shown by *Symphynota* and *Anodonta* (*A*, *B* and *C*) in which the shell outline is a distinguishing feature.

BEHAVIOR AND REACTIONS OF GLOCHIDIA

At the time of spawning the glochidia, already freed from the egg-membranes and more or less loosely held together in slimy strings, are discharged at irregular intervals through the exhalent siphon. Being heavier than water, they sink rapidly to the bottom, coming to rest with the outer surface of the shell directed downward and the valves gaping widely apart. The belief was formerly general that they "swim" about by rapidly opening and closing the valves, after the manner of *Pecten*, and in spite of frequent denials by Schierholz ('88), Latter ('91), and others, the same statement is still occasionally encountered. In the recent volume on Mollusca in the *Treatise on Zoölogy*, edited by Lankester, this inexcusable error is repeated. "The glochidia," we are again informed, "swim actively by clapping together the valves of the shell," (p. 250). They are on the contrary, as is now well known, entirely incapable of locomotion and remain in the spot where they happen to fall, although it is true that they may exhibit from time to time spasmodic contractions of the adductor muscle which cause the valves to snap or wink, each contraction being immediately followed by relaxation and opening of the shell. These movements of the valves, however, are never so vigorous as to cause the glochidium to move from place to place in the water.

The glochidia remain in this helpless situation until they die, unless they happen to come in contact with the host on which they pass through the post-embryonic development as parasites. The stimulus which causes the contraction of the muscle and results in attachment to the host is in the case of hooked glochidia, a mechanical one. It may be readily imitated and glochidia of

this type made to grasp firmly the point of a needle or the edge of a piece of paper by simply touching them between the open valves. When once closed in this manner they do not relax but remain attached to the object until they die.

Electrical and chemical stimuli may also under proper conditions bring about permanent closure of the shell.

The following statement made by Latter (*l. c.* p. 56) has been frequently quoted, especially in text-books, but it has apparently never been verified or disproved. "The Glochidia," he says, "are evidently peculiarly sensitive to the odor (?) (*sic*) of fish. The tail of a recently killed Stickleback thrust into a watch-glass containing Glochidia throws them all into the wildest agitation for a few seconds; the valves are violently closed and again opened with astonishing rapidity for 15–25 seconds, and the animals appear exhausted and lie placid with widely gaping shells—unless they chance to have closed upon any object in the water (*e. g.* another Glochidium), in which case the valves remain firmly closed." Although it is not stated that the tail which caused such a commotion among the glochidia had been cut off from the fish, it is probable that such was the case. We have repeatedly tested glochidia in the same manner both with fins and gills of different fishes, and, provided that a bleeding surface is not brought in contact with the water containing the glochidia, absolutely no response on the part of the latter takes place. The result, however, is much as Latter describes if a little of the fish's blood gets into the water in the neighborhood of the glochidia, except that our experience has shown that after snapping for a few seconds they come to rest in permanent closure. It, therefore, seems possible that the contractions seen by Latter were due to the introduction of some blood with the tail of the fish, as otherwise agitation of the glochidia under similar conditions has not been observed by us.

Since the hooked and hookless glochidia, whose reactions to blood and to certain salts we have examined, behave somewhat differently, they are referred to separately below.

Reactions of hookless glochidia

The glochidia of *Unio complanatus* were used in the following experiments. When a small drop of blood of either the killifish, *Fundulus diaphanus*, or the white perch, *Morone americana*, was placed over the glochidia contained in a small amount of water in a watch-glass, the effect was immediate and very striking. Every glochidium was thrown into rapid and violent contractions, alternating with relaxations, the edges of the valves either quite or nearly touching with each snap. Where the stimulus was strongest, that is, immediately under the drop of blood, the glochidia exhibited two or three strong contractions and then remained closed, but proceeding outwards to zones of diminishing intensity, the snapping occurred intermittently for from ten to fifty seconds. Here the contractions were quite rapid at first, one or two every second, but soon the intervals became longer, until finally the activity was ended by the closure of the valves. In some cases it was observed that after the first few snaps the muscle did not completely relax and each subsequent contraction caused the valves to describe a shorter arc.

Since the hookless glochidia are essentially gill parasites and, when taken into the mouth of the fish, lodge among the gill-filaments, abrasions of the delicate epithelium covering the latter always occur and produce more or less extensive hemorrhage from the blood capillaries. It is, therefore, evident that blood exuding in the neighborhood of the glochidia must have the same effect as in our experiments, and, by causing vigorous contractions of the adductor muscle, be efficacious in bringing about a firm and permanent attachment to the filaments. And, furthermore, since glochidia of the hookless type only occasionally exhibit spontaneous contractions, and, unlike the hooked forms, respond either not at all or only quite sluggishly to tactile stimuli, the action of the blood upon them must play an important part in securing their attachment to the gills.

A series of experiments was also undertaken for the purpose of determining the reactions of the glochidia of *Unio complanatus* to solutions of several different salts, a brief account of which

may be given here. Diluted sea-water and solutions, varying in strength from 0.5 to 1.0 per cent, of NaCl, K₄Cl, KCl and NH₄Cl had exactly the same effect as fish's blood, although the intensity of the reaction varied somewhat in certain cases. Weak solutions of MgCl₂ and MgSO₄, however, as was to be expected, inhibited contractions, and glochidia, after treatment with these salts, could be killed in an expanded condition, if allowed to remain in the solutions for a sufficient length of time.

Reactions of hooked glochidia

The larvæ of *Symphynota complanata*, which are provided with stout hooks and as a rule find permanent lodgment only on the fins and other external parts of the fish, were used in studying the reactions of the hooked type of glochidium. In several respects they differ from the hookless forms. When removed from the marsupium and placed in water, they exhibit spontaneous contractions which occur at irregular and rather long intervals, and this irritability may continue in the laboratory for a day or two, or until the glochidia begin to disintegrate. Under such conditions the valves are only partially closed at each contraction of the muscle, which, moreover, is never strong enough to bring the points of the hooks into contact. It is followed at once by relaxation of the muscle and the shell remains widely open until the next snap occurs.

Hooked glochidia, in marked contrast with the behavior of the hookless forms, respond very actively to tactile stimuli, and, as has been stated, close completely and immediately when touched with any object. This reaction must be the main factor in bringing about their attachment to the fish's fins, when they are brushed over by the latter while lying on the bottom. With glochidia like those of *Symphynota complanata* the mere contact is sufficient to produce complete closure of the valves, and, whether they are exposed to the fish's blood, or not, attachment is possible as a result of the tactile stimulus alone. They do react to blood, however, and exhibit a few successive contractions, from five to fifteen, before final closure, but the way in which the response

occurs is quite different from that shown by the glochidia of *Unio complanatus* under similar conditions. Instead of being thrown into violent and rapid snapping, the valves closing and opening alternately, there is only partial recovery after each contraction, while the valves are brought closer and closer together by a series of short jerks. The final act of closing is interesting. As soon as the points of the hooks touch, the contraction of the adductor muscle becomes continuous and the hooks are slowly bent inwards against each other. Under the steady pressure exerted by the muscle, aided probably by the action of the myocytes, which have been described by Schmidt ('85 b), the spines on the outer surface are apposed and the hooks turned in completely between the valves, the margins of which are brought together, if no object intervenes. It will be readily understood that, owing to the turning in of the hooks, the spines are pressed into the fish's tissues, when attachment to the host takes place, and a firm hold is thereby secured.

When the glochidia of *Symphynota complanata* were exposed to salt solutions, the contractions produced were of the kind just described. KCl, KNO₃, and NH₄Cl in solutions of 0.5 to 1.0 per cent caused a few successive jerks, the contractions being more vigorous and closure occurring sooner with the stronger solutions. NaCl and Na₂C₂O₄ in the same strength acted less energetically, and it was necessary to use a two per cent solution to produce the same effect as was obtained with the weaker solutions of potassium and ammonium salts. A 0.5 per cent solution of CaCl₂ produced no contractions, while a 1.0 per cent solution after a latent period of fifteen minutes caused either partial or complete closure of the valves. MgCl₂ and MgSO₄, in solutions of 0.5 and 1.0 per cent, inhibited contractions, and when the glochidia were allowed to remain in them they finally died in the expanded condition. When the Mg salts, however, were used in stronger solutions closure of the valves occurred after a few spasmodic contractions.

THE PARASITISM

The only infections which we have ever observed in nature were of *Anodonta grandis*, found in the month of November, infecting the roach (*Abramis crysoleucas*), German carp (*Cyprinus carpio*), yellow perch (*Perca flavescens*), blue-gill sunfish (*Lepomis pallidus*), rock bass (*Ambloplites rupestris*) and crappie (*Pomoxis annularis*), collected from the Mississippi River at LaCrosse, Wisconsin. At that time, we handled over 25,000 fish and from the finding of these glochidia upon a majority of the fish taken at random for examination in connection with our own infections it seemed that a large proportion of the whole number were lightly infected with this glochidium. By actual count, we found from one to twenty upon a fish and they were all in about the stage shown by fig. 23. These fish had been recently collected from sloughs, similar to those in which we found many individuals of *A. grandis* with ripe glochidia, and this probably represented a typical infection under natural conditions, where we may be sure that maximum infections never obtain.

Following the methods of artificial infection as practiced since the work of Braun ('78) and Schmidt ('85), we have obtained unlimited material by confining the fish in small receptacles to which the glochidia have been added by washing from the gills of the clams. It is only necessary to see that the glochidia are so distributed in the water as to come in contact with the proper part of the fish, and in most cases, to guard against over, rather than under, infection. Active fish, such as the rock and the large-mouthed black bass, are very favorable for gill infections, since they keep the water so well agitated that the glochidia hardly settle to the bottom at all, while their strong respiratory movements draw the suspended glochidia continually against the gills. Fish like the crappie, which when undisturbed move about quietly and whose respiratory movements are less vigorous, must have the water stirred to keep the glochidia suspended, or so shallow that the fish are always near the bottom. The smaller gill slit of the crappie is another factor which makes for a very light infection in fish under two inches in length, since the glochidia

reach the gills by way of the mouth and not from the opposite direction. For fin infections, sluggish fish like the German carp need little attention, and the darters (*Etheostoma coeruleum* spec-tabile) which habitually rest upon the bottom for considerable periods, become quickly loaded with glochidia upon both fins and gills, though as we shall see, the latter appear to be particularly adapted for ridding themselves of the entire infection.

Infections with hooked glochidia

For the infections with hooked glochidia, we have used principally *Anodonta cataracta* from Falmouth, Massachusetts, the species studied by Lillie ('95). With these, we have, infected German carp under six inches in length and, unless otherwise stated, the following account refers to this combination which gives typical results. A smaller number of infections made with *Symphynota complanata* and *S. costata* upon carp and other fish are referred to in a supplementary manner. The glochidia of *A. cataracta* become attached in large numbers to the fins (Figs. 7-11 and gills of the carp. They are also found upon the other external parts which offer the condition of a soft scaleless epithelium like that of the fins; thus the region about the anus, the edge of the operculum, the lips and, in very heavy infections, even the soft area of the ventral surface between the mouth and pectoral fins may become heavily loaded. Within the mouth cavity, the gill-filaments and also the gill-bars and rakers become well covered. The glochidia which attach to these mouth parts do not remain. for, though the fish may be carrying many of their fellows upon its external parts, in about one week after the infection all glochidia have disappeared from the gill-filaments, which then become as clean as though never infected. There is some chance of a scattering of glochidia remaining upon the other internal mouth parts, for such specimens are occasionally seen well embedded and in advanced stages of their metamorphosis, but in the main, these parts also will become free of glochidia.

The general distribution upon the individual fins may be seen by reference to figs. 7-11, which show how great a proportion

of the glochidia become attached to the fin margins. If a fish is carefully watched, as its slight movements stir up the glochidia during the infection, the latter are seen continually falling upon the upper faces of the pectoral and pelvic fins. They may even be collected with a pipette and heaped upon a motionless pectoral, remaining there for some minutes without more than an occasional specimen becoming attached. The margin of the fin is so much more favorable for attachment, that it is often thickly set with glochidia, when none are found upon the fin surface, and this despite the fact that glochidia must, during infection, strike against the surfaces of the fins, many times for every time that one of them comes in contact with a fin margin. It is, therefore, the fin margin for which this glochidium is best suited, and once fastened there, it is almost certain to remain and become overgrown. When a specimen does fasten to the surface, it probably gains its hold by catching upon one of the ridges formed by the fin rays, for the hooks could hardly be used upon a perfectly flat surface. Glochidia sometimes hold to the surface of a fin by a shred of tissue, under which their hooks have caught and remain there after all their fellows (fig. 11) are completely overgrown, only to be torn off later without having caused any noticeable hypertrophy of the fin tissue. Figs. 11 and 14b show that glochidia may become overgrown either flat against the surface, or upon edge, and fig. 12 shows a young mussel leaving a surface attachment after a parasitism of seventy-four days.

The distribution of the glochidia to the several fins is determined solely by the number likely to be brought in contact with a given part of the body. Those fins which brush against the bottom are always the more heavily loaded and the numbers elsewhere depend upon the extent to which the glochidia are kept suspended on the water.

Optimum infections, as we shall term those which are close upon the limit of the number of glochidia which a fish can safely bring through the metamorphosis, often show the glochidia very closely set one after another, as in fig. 11, and several hundred may be safely carried by a fish three or four inches in length. Prolonged exposure causes so heavy an infection of the margins

(fig. 9) that the fin tissue appears unable to overgrow the mass of glochidia and they then remain attached without overgrowth for a week or more. Fig. 10 shows how, in a part of the fin having no overcrowding, normal embedding occurred; while in the more crowded areas the glochidia were still uncovered even seven days after infection. In the region of the middle upper margin of this figure, it would seem that the overgrowth might well have taken place; for many cases, like fig. 11, have been observed in which glochidia as closely set were properly embedded. The failure of overgrowth in this region is probably due to the presence immediately after infection of a greater number of glochidia, many of which have since been detached. In all cases of this kind, a smaller number will finally become embedded than in an infection where the fin has received more nearly the optimum load (figs. 7, 8 and 11), for the great majority drop off when the fin becomes so mutilated that bacterial or fungus infection sets in. These over-infections sometimes cause so much hypertrophy that the fins become lumpy and the rays so much drawn together that it is impossible for the fin to spread out normally. Often, the fins are raw and bleeding for some days and show red areas within where the blood vessels have become abnormal. The fish are likely to die from this, or from the similar injury to their gills, and these over-infections are unsatisfactory, if one wishes to bring through their parasitism the maximum number of glochidia per fish.

The steps in the implantation of the glochidium by an overgrowth of the fish's tissue may be seen in figs. 7-11 and 20-23. Figs. 7 and 20 show the glochidium three and one-half hours after the fish was removed from the infection. Most of the glochidia have bitten deep enough in from the margin to have a good hold for their hooks. The beginning of the hypertrophy appears as a faint mass of tissue, seen with its nuclei in the detailed fig. 20. At the end of twelve hours, the overgrowth is well advanced and sometimes, as fig. 21, shows different stages even in neighboring glochidia. The ragged edge of the host's tissue rises up crater-like about the glochidium, meeting above in a delicate mass, the nuclei of which are shown. Fig. 8 shows that, in twenty-

four hours, most of the glochidia are more than half covered, whether upon the edge or the surface of the fins. At the end of thirty-six hours, optimum infections of the carp show all the glochidia, which have obtained a proper attachment, well embedded, and after this the only external change is a slight increase in opacity which renders the internal structure of the glochidium less distinct. Some of our infections show embedding in as short a time as six hours (Symphynota) and Harms ('09) gives ten to twelve hours, as the time which he observed in Anodonta, so the time given for the figures is the maximum for hooked glochidia which have been well located. Glochidia upon the fin surface become embedded in a similar manner and are then in a very secure position. (Figs. 11 and 14b.)

Infections with hookless glochidia

Following these same methods of artificial infection, we have made more extensive experiments upon the parasitism of the hookless glochidia, which is the only type found in our commercial mussels. Species of the genus *Lampsilis* (*ligamentinus*, *rectus*, *anodontoides*, *ventricosus*, *subrostratus* and *luteolus*) have been the most used, but infections have also been made with several species of *Quadrula* and one of *Unio*. The list of fish employed as hosts is also more extensive and we are, therefore, able to make statements which we know to be of wider application than those made for the hooked forms.

When the same fish is used, the results for the several species of *Lampsilis* are very uniform and we can thus discuss the parasitism of this genus as a whole; but we do not find the same mussel giving uniform results with all species of fish. The glochidia of this genus have been used successfully for the infection of blue-gill sunfish, yellow-perch, crappie, large-mouthed black bass (*Micropterus salmoides*), rock bass, the red-spotted sunfish (*Lepomis humilis*) and the green sunfish (*Apomotis cyanellus*). As with the hooked glochidia, the infections have all been made upon fish under six inches in length, upon which these glochidia remain in numbers only on the gill-filaments, though during infection many

become attached to and even embedded upon the fins and other external parts. Harms ('08) concludes that the hookless type persists in much greater numbers on the fins of small than of large fish and that the hooked type will survive upon the gills if large enough fish are used, and it is doubtless true that the size of the gills and fins is an important factor in determining the place of attachment for each type, since the hookless form is only adapted for holding to a delicate surface like the gill-filament, or a fine fin while the hooked seem likely to be easily torn from just such a surface. When the hookless form does once become established upon an external part, it will develop there without mishap as shown by a figure of a hooked and hookless glochidium developing side by side upon the margin of a fin (fig. 23). Within the mouth cavity, the glochidia will attach to the gill-bars and rakers if these parts are covered by a sufficiently delicate epithelium, but upon the gill-filaments they are always found in the greatest numbers. In most of our infections the filaments are the more heavily infected toward their outer ends (fig. 19), but the distribution varies somewhat with the species of fish. For example, successful infections of rock bass with *Lampsilis ligamentinus* show about seven glochidia upon the distal third of the filament to one upon the proximal two-thirds; of large-mouthed black bass about three to one; and of yellow-perch about one and a half to one; differences which are probably caused by some particular configuration of the mouth cavity which causes the glochidia to fall more upon one part of the filaments than another.

In a fish which will carry a given glochidium successfully, overinfection of the gills is easily accomplished and easily fatal, but the species of fish differ greatly in the amount of infection they are able to carry without serious mortality. In one of our most successful combinations (rock bass + *Lampsilis ligamentinus*). fish four inches in length were estimated to be carrying in the neighborhood of 2500 glochidia, an average of more than two for every filament of the gills and yet there was almost no mortality among the fish. In this case the success of so heavy an infection is perhaps explained by the distribution of the glochidia upon the gill-filaments, for we found by count that there were about seven

near the tips to one on the sides and thus the greater part of every filament was left unchanged and in full functional condition, while in other infections (large-mouthed black bass + *L. ligamentinus*), where a much greater proportion of the glochidia were upon the sides of the filaments, the mortality of the fish was very heavy, although the amount of infection was much less. A gill of the latter fish, from a lot lightly infected with these glochidia is shown in fig. 13. The number estimated for this fish, which was four inches long, being only 450, is distinctly less than the optimum.

Implantation upon the filaments occurs in a manner similar to that of the hooked glochidia upon the external parts, but much more rapidly. Figs. 15, 16, 17 and 18 show the appearance at 15 minutes, 30 minutes, 1 hour and 3 hours after infection, and our observations showing that the cyst is completed within from two to four hours, agree with what Harms ('09) has found for gill infections. The proliferation will even continue after the gill has been cut from the fish and placed in a watch glass for observation under the microscope. An immediate result of the cyst formation is the obliteration of the lamellæ upon either side of the gill-filament, which thus becomes smooth and slightly swollen in the vicinity of the glochidium. Figs. 13 and 19 show the general and detailed appearance of the cysts and the diversity in the angles at which the glochidia are attached.

The older statement that the hooked glochidia are fin and the hookless gill parasites finds, therefore, confirmation from our work though it would be better to say that the hooked attach most successfully to large strong margins like those of the fins while the hookless to soft and fine filamentous structures like the gills in fish of moderate size. The reaction of hookless glochidia to blood, with respect to the part it plays in attachment, has already been described.

Susceptibility of fish to infection

The susceptibility of different fish to infection is a matter which we think has not been sufficiently considered by any previous

investigators for we seem to have evidence that some fish are much less susceptible than others and that with such fish any considerable infection is an impossibility, the most striking instances of this being the German carp, minnows and darters.

In the case of the carp, we have a fish admirably suited for carrying the hooked glochidia of *Andonta* and *Symphynota*, but we have never been able to secure a successful infection of the gills of the carp with the hookless glochidia of the genus *Lampsilis*. The disappearance of the hooked glochidia *Anodonta* and *Symphynota* from the gills of the carp, as previously mentioned, is explicable upon the grounds given in our consideration of the reasons for the survival of the two types of glochidia upon the fins and gills respectively but any such explanation is impossible when applied to this disappearance from both gills and fins of the glochidia of *Lampsilis*.

With minnows (*Notropis cayuga* and *N. lutreusis*) two to four inches in length, we have not been able to secure any considerable infection with the glochidia in *Symphynota complanata*, for, though they will attach in large numbers during infection, they all drop from the fins and gills within a few days. The fin of these minnows, is much more delicate than that of the carp and the explanation is perhaps that so large a glochidium is easily torn away; but the large mouthed black bass has hardly a delicate fin, and for it we have records of infection where no glochidia of *S. complanata* attached during an exposure sufficient for the attachment of many to the gills. In this case, the extreme activity of the fish must be considered as a factor which might keep the hooked glochidia from attachment to the fins.

With darters, (*Etheostoma coeruleum spectabile*) one and one half to two inches in length, there appears to be an almost complete immunity against the permanent attachment of *Lampsilis* glochidia, for though they may fasten so thickly to the fins that many fish are killed within the first day after the exposure, the fish which survive this will slough off considerable portions of the fins and within a week show only the healed and regenerating parts as an indication of their recent experience. Such cases as these are of great importance and should be followed up to deter-

mine whether the simple mechanical causes of over-infection, delicacy of fin, or configuration of the mouth parts can give a satisfactory explanation; or whether the histological changes which the fish is capable of, under stimulation, by the glochidium, must be regarded as the causes of its immunity. We have not yet carried out sufficiently rigorous experiments to feel sure that the simpler explanations can be excluded. In any case, it is interesting that fish like the minnows and darters, which live close to the bottom, are not likely to become heavily infected by some of our most common glochidia.

Duration of the parasitism

The duration of the parasitism appears, to be influenced very considerably by the temperature as stated by Schierholz ('88) and Harms ('07-'09). Our records show a difference in the time, spent by a given glochidium upon the same fish, which varies with the season. For example, we have records of *Symphynota complanata* completing its metamorphosis and leaving the fish in from ten to fourteen days during December, and others in which the parasitism, begun March 25th, was continued for about forty days. *S. costata*, in an infection begun during January, remained upon the fish for upwards of seventy days. Infections with *Lampsilis glochidia* have shown a variation in the duration at the temperature of the laboratory (16°-20° C.) of from 36 to 14 days. The glochidia of *Unio complanatus* and of *Quadula plicata* in July infections gave a period of from twelve to fifteen days. In all these cases, the temperature is probably the cause of the differences, as Harms believes.

HISTOLOGICAL CHANGES IN CYST-FORMATION

As has been described, the glochidium attaches itself to the fish by closing its shell firmly over some projecting region which can be grasped between the valves, like the free border of a fin or the gill filament. In so doing, a portion of the epithelium and underlying tissue, including blood vessels and lymphatics, and

varying in amount with the extent of the "bite," becomes enclosed within the mantle space of the glochidium. This tissue early disintegrates into its cellular constituents, which are taken up by the pseudopodial processes of the larval mantle cells, and as Fausek ('95) has described, are utilized as food during the early stages of metamorphosis. In fig. 37, drawn from a glochidium six hours after attachment to a fin, the disintegrated tissue, consisting of loose epithelial cells, blood corpuscles, and fibers which lie scattered in the mantle cavity, is seen in the process of being ingested by the mantle cells. Fig. 38 shows a later stage, twenty-four hours after attachment, in which the detritus has been entirely taken up, and the mantle cells are now heavily charged with food material.

Almost immediately after attachment proliferation of the epithelium begins as the initial step in the formation of the cyst which eventually encloses the entire glochidium. The overgrowth of the larva has been described by Fausek ('95) and Harms ('07, '09) as a healing process on the part of the fish's tissues, resulting from the irritation caused by the wound. The proliferation starts around the line of constriction produced by the pressure of the edges of the valves on the epithelium, and, since the glochidium lies between and prevents the immediate closure of the lips of the wound, the extending epithelium is forced to slide up over the surface of the shell on all sides, until the free margins meet and fuse over the back of the larva, as may be understood by reference to figs. 15-18 and 20-22.

So rapid is the overgrowth, especially in the case of implantation on the gills, that it would seem that something more than the mere mechanical irritation produced by the glochidium is concerned in causing the proliferation of the epithelium. We have, therefore, carried out a series of experiments with a view to determining whether or not, a chemical stimulus is provided by the larva, and by using various methods have studied the action of glochidial extracts on the epithelium of both fins and gills. The results have been entirely negative, although the question has by no means been settled by the experiments which have been thus far attempted. By further improvements in the technique,

some of the difficulties involved in the investigation, which is still in progress, may be overcome.

The histological changes taking place in the epithelium during the formation of the cyst have been studied in this laboratory by Miss Daisy Young, and as her results will soon be published in detail, only a very brief reference will be made to the subject here. We are indebted to her for the use of six of her drawings which are reproduced in this paper in figs. 33-38, in order to illustrate the essential points involved in the cellular changes occurring during implantation.

Fig. 37 shows an early stage, 6 hours after attachment, in the formation of the cyst on the fin. The proliferation begins in the neighborhood of the constriction where several mitoses may be seen in the figure, and this seems to be the region of active growth and multiplication of cells. As the cells at this level increase in number, they appear to push those lying above them up over the outside of the shell, so that the actual covering of the glochidium is due largely to this mechanical gliding of the epithelium over its surface. Sections give no evidence at all of amitotic division, while mitoses are generally abundant in the region of active proliferation. Fig. 38 shows a case of complete implantation on a fin in 24 hours. The wall of the cyst is seen at this time to be quite thick, but it usually becomes thinner later on as the cells composing it flatten down.

In figs. 34, 35, and 36, a series of stages are represented in the formation of the cyst on gill-filaments, taken at 15 minutes, 30 minutes, and 3 hours, respectively, after attachment. Fig. 34 shows the very beginning of the proliferation and the presence of two or three mitotic figures just below the glochidium and near the raw edge of the constricted epithelium. A little coagulated blood is seen on the surface of the shell and around the lips of the wound, showing how intimately the larva comes into contact with blood at the time of attachment, as referred to above. A large mass of the fish's tissues, including portions of blood vessels, is also shown in the figure enclosed within the mantle chamber. At the next stage (fig. 35), the pushing-up of the epithelium has made considerable progress; several mitoses appear in the

section, while a few loose epithelial cells are sloughing off at the edges of the growing cyst—a not infrequent occurrence during the early stages of implantation. Fig. 36 shows the completion of the process, and the glochidium is now, after three hours in this case, entirely enclosed within its epithelial covering.

LIBERATION FROM THE CYST AND POST-LARVAL STAGES

In about one week as a rule after attachment, the wall of the cyst begins to assume a looser texture, the intercellular space becoming infiltrated with lymph, and from this time on to the end of the parasitic period there is little further change in its structure.

Before liberation of the young mussel, the valves open from time to time and the foot is extended. By the movements of the latter the cyst is eventually ruptured, its walls gradually slough away, and the mussel thus freed falls to the bottom. Fig. 33 shows an early stage in the breaking up of the cyst which is seen to be coming off in patches on one side. Portions of the wall of the cyst often adhere to the shell after liberation, while, if the young mussel has hooks, it may hang for a time by shreds of the fin in which the hooks are embedded, as seen in fig. 12.

We have not succeeded in keeping the young mussel alive in the laboratory for a longer period than six weeks. From the first they are very active and creep about in a dish by stretching out the foot, securing a hold by flattening the distal end against the bottom, and then drawing up the body after the fashion of other small lamellibranches. Fig. 29 gives an excellent illustration of the various positions assumed as they crawl about, and also shows the extent to which the shell has developed beyond the margins of the glochidial valves by the end of the first week of free life.

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PLATE 1

EXPLANATION OF FIGURES

- 1 Gravid female of *Ptychobranchus phaseolus*. $\times \frac{3}{4}$
- 2 Gravid female of *Lampsilis subrostratus*. Natural size.
- 3 Gravid female of *Obliquaria reflexa*. Natural size.
- 4 Gravid female of *Quadrula ebena*. $\times \frac{1}{2}$.
- 5 Gravid female of *Lampsilis rectus*. $\times \frac{1}{2}$.
- 6 Gravid female of *Symphynota complanata* $\times \frac{1}{2}$.

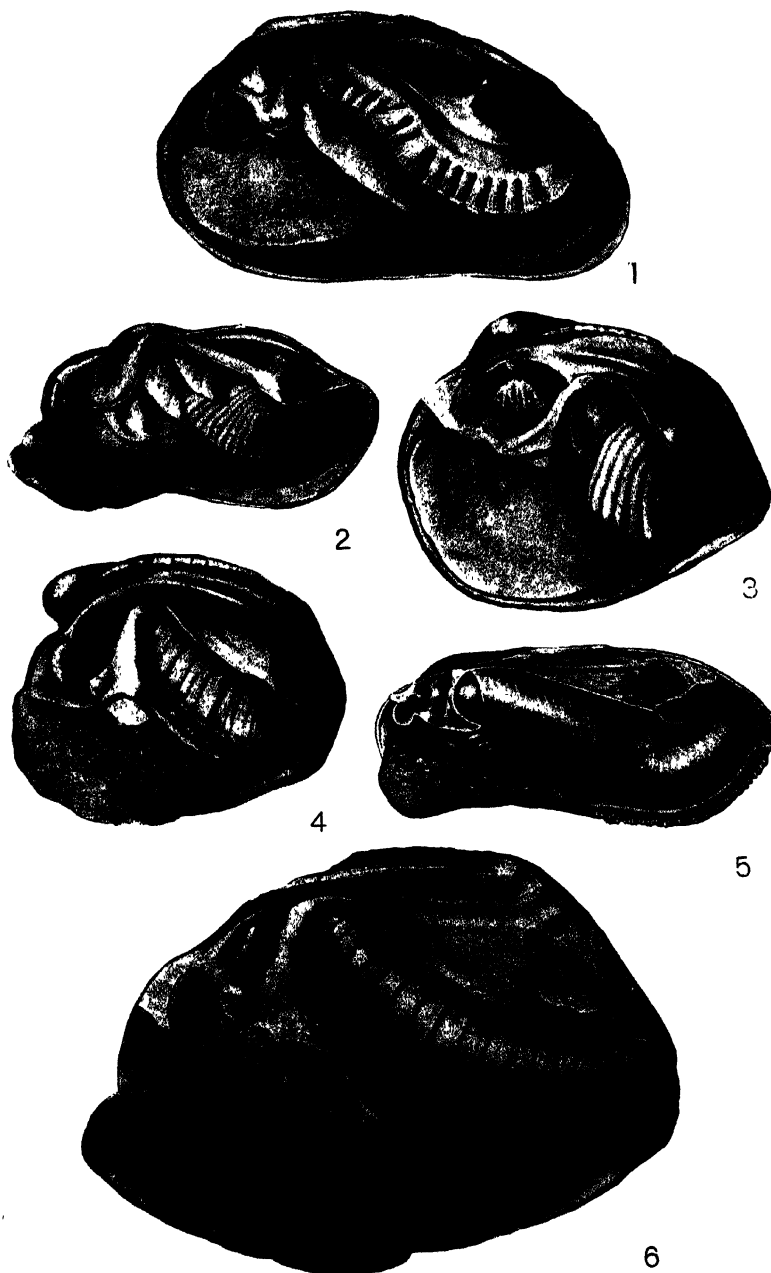


PLATE 2

EXPLANATION OF FIGURES

7 Pectoral fin of a carp, about 3 inches long, 3½ hours after infection with glochidia of *Anodonta cataraeta*. An optimum infection.

8 Ventral half of the tail of a carp, as above, 24 hours after infection. An optimum infection.

9 Tip of an over-infected fin, as above, 12 hours after infection. Showing no appreciable over-growth because of the crowding. The shadows represent glochidia upon the under surface.

10 Fin, as above, 7 days after infection. Showing the complete failure to embed in all places where the glochidia are greatly crowded. See explanation in the text p. 106 of the conditions along the upper margin.

11 Fin, as above, 36 hours after infection. Showing the complete overgrowths of all glochidia which have secured the proper attachment.

12 Young of *Symphynota costata*, leaving the fin of a carp after a parasitism of 74 days.

13 Anterior gill of a black bass infected with *Lampsilis ligamentinus*, showing distribution upon the gill as a whole and the appearance of the cysts.

14a Glochidium of *A. cataraeta* upon fin of carp. Developing normally after a shift of 90 degrees from the position first taken.

14b Two glochidia of *Anodonta cataraeta*, overgrown after 36 hours upon surface of a carp's fin.



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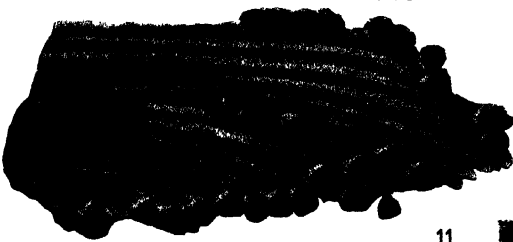
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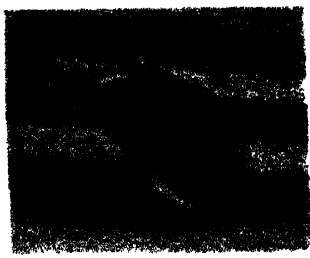
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PLATE 3

EXPLANATION OF FIGURES

15, 16, 17, and 18 Stages in the formation of the cyst about a hookless glochidium (*Lampsilis ligamentinus*) upon a gill filament of the black bass. Taken at 15 min., 30 min., 1 hour, and 3 hours, after infection. The transverse lines on the filament are the lammellæ.

19 Part of a gill of black bass infected by *L. ligamentinus*. Showing the distribution and orientation of the glochidia in an infection above the optimum for this fish. Only the layer of filaments toward the observer is shown.

20 Glochidium of *Anodonta cataracta* upon fin margin of carp. 3½ hours after infection. Proliferation of the cyst just beginning.

21 Glochidia, as above, upon fin margin of carp. Showing different stages of cyst proliferation, even in neighboring glochidia.

22 Glochidia, as above, 24 hours after infection.

23 Hooked and hookless glochidia (*A. grandis* and *L. rectus*) embedded and developing on a fin margin. The former received in nature and therefore older than 28 days, which is the age of the latter.



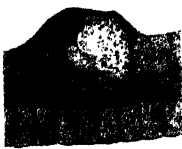
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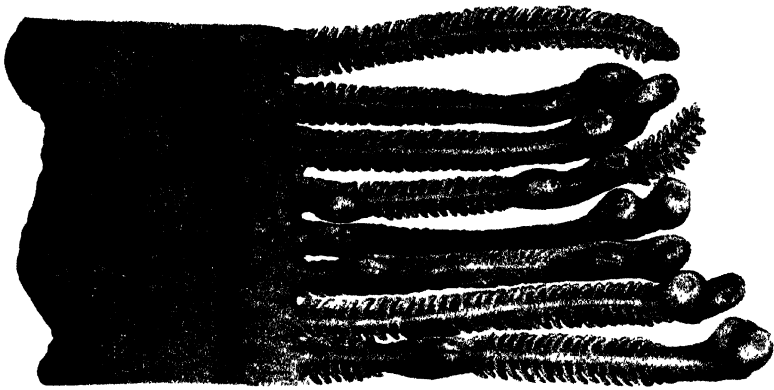
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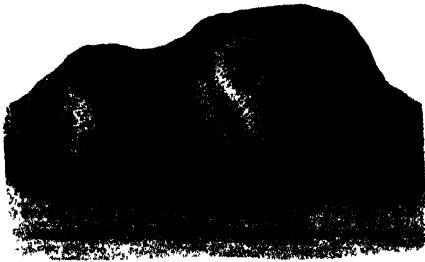
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PLATE 4

EXPLANATION OF FIGURES

24 Detail of a conglutinate of *Lampsilis ligamentinus*. The glochidia, still enclosed in the membranes, are less crowded together than those of fig. 28, and are embedded in a mucilaginous matrix.

25 Glochidium of *Lampsilis (Proptera) alatus*.

26 Three conglutinates of *Obliquaria reflexa*, removed from the marsupium $\times 2$.

27 Two conglutinates of *Lampsilis ligamentinus*, removed from the marsupium. One is shown from the flat surface, the other on edge. $\times 2$.

28 Detail of a conglutinate of *Obliquaria reflexa*, showing the membranes closely pressed and adhering together.

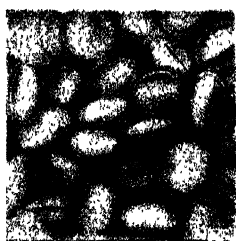
29 Young mussels of *Lampsilis ligamentinus*, one week after liberation from the fish, showing various positions assumed in crawling, the ciliation of the foot, and the new growth of shell.

30 Transverse section of two water-tubes of the gravid outer gill of *Quadrula ebena*, showing the glandular epithelium on the interlamellar junctions and two embryos in an early cleavage stage.

31 Transverse section of a single water tube of the marsupium of *Lampsilis alatus*, showing the much greater size of the tube than in the last figure, which is drawn under the same magnification. Several embryos in an early cleavage stage are seen in the tube.

32 Highly magnified section of a portion of the glandular epithelium lining a water-tube of the marsupium of *Quadrula ebena*, showing the large mucus cells and several nuclei of leucocytes (1) with which the epithelium has become infiltrated.

33 Section of young mussel, *Unio complanatus*, 11 days after attachment of glochidium to gill-filament, still in cyst which is beginning to break away



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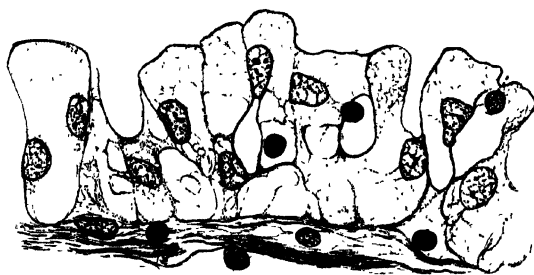
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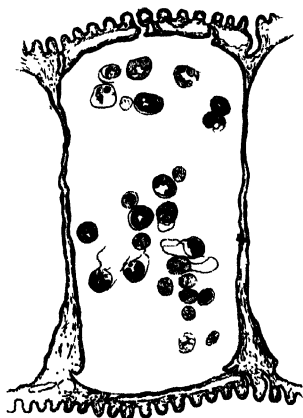
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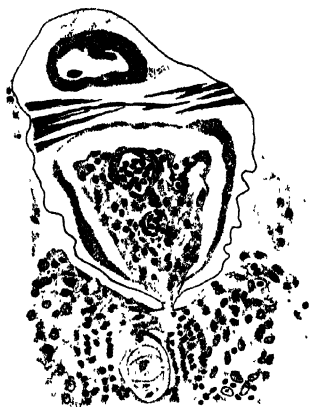
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PLATE 5

EXPLANATION OF FIGURES

34-36 Sections of glochidia of *Lampsilis ligamentinus*, taken 15 minutes, 30 minutes, and 3 hours, respectively, after attachment to gill filaments. In 34, the proliferation of epithelium is just beginning; in 35 it has made some progress; and in 36 formation of the cyst has been completed. In the first two figures several mitoses are shown in the epithelium where multiplication of cells is taking place.

37-38 Sections of glochidia of *Symphynota complanata*, 6 and 24 hours, respectively after attachment, showing two stages in cyst-formation on fins. In 37 the cellular detritus is being ingested by the larval mantle cells, while in 38 this process has been completed. Mitosis in the cyst wall is shown in both figures.



34



37



35



36



38

THE NEMATOCYSTS OF EOLIDS¹

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ELEVEN FIGURES

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INTRODUCTION

The note on the nematocysts of nudibranch molluscs which I published in the Circular of the Johns Hopkins University in March, 1903, was the outcome of suggestions made by Professor Brooks, not only as to the possible origin of eolidian nettles, but as to the advisability of presenting at once the evidence at that time available. The necessity of teaching in summer schools during the only months when work on eolids is possible has caused the delays which have robbed me of the pleasure of offering my results prior to partial anticipation by others, and in time for Dr. Brooks to see them.

¹ From the Zoölogical Laboratory of the University of Michigan, 

In addition to the rhinophores (fig. 1), with their parallel rings, and the mobile foot-tentacles, sensitive to touch, eolids possess numerous dorsal appendages, or cerata. These structures, often banded distally, and tipped with blue, purple, grey, or white, occur in clusters symmetrically distributed on the sides of the body. When, as is frequently true, the cerata are very abundant, their distribution seems to be uniform; nevertheless each ceras belongs to a particular group that radiates from an enlargement on the side of the body. These swellings are regions of prolifer-



Fig. 1 Typical eolid, showing rhinophores with parallel ring-like swellings; foot-tentacles, and cerata of various sizes radiating in groups from lateral enlargements.

ation and from them arise new appendages in such manner that the oldest in each collection is nearest the sagittal axis of the animal.

A median section through such a ceras (fig. 2), shows an ectodermal covering (*ECT*), indistinct in cell-boundaries, and rich in mucous glands. Inside this are bundles of longitudinal and circular muscles (*MUSC*), enclosing a more or less capacious duct whose distal pore (*CNDP*), completes a passageway from the lumen of the liver to the outside. This duct, lined proximally with hepatic cells, then with ciliated epithelium, leads distally into a cnidophore filled partially or completely with cells or cysts containing nettles. The basal entrance into the cnidophore is guarded by a sphincter (*SPH*).

The morphology of the cerata is so thoroughly understood, that there is no need to add anything to the writings of Davenport ('93), Hecht ('96), or Krembrow ('02). The origin of the nematocysts, however, is still a subject for debate since many

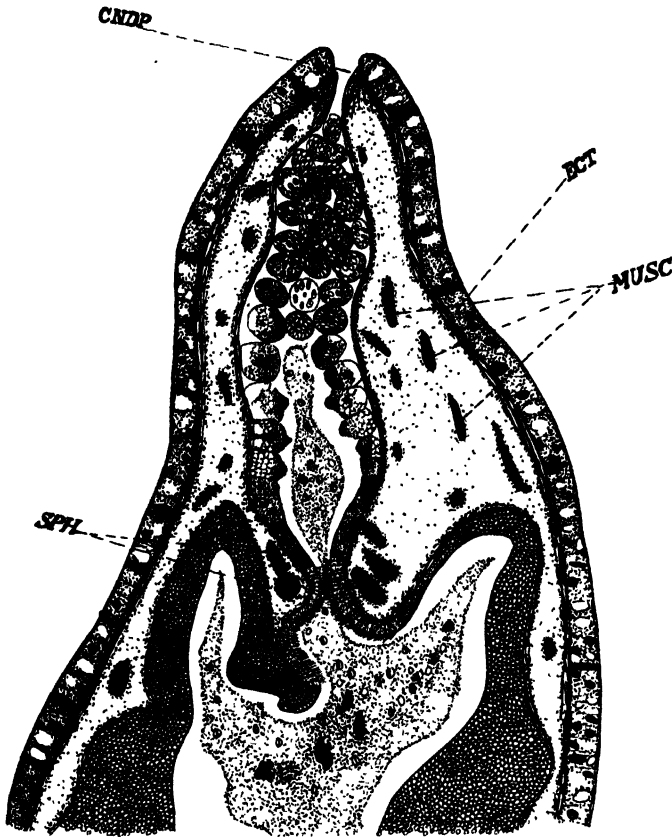


Fig. 2 Diagrammatic median longitudinal section through ceras showing complete cnidophoral system. CNDP., cnidopore; ECT., ectoderm; MUSC., longitudinal and circular muscles; SPH., sphincter.

biologists remain incredulous despite the published evidence. Grosvenor ('03) pointed out that this was the attitude assumed by some authorities toward T. Strethill Wright, who in 1858 read, before the Royal Physical Society of Edinburgh, a paper in which he attempted to show that the nematocysts of eolids are

derived from their hydroid prey. According to Wright, the possibility of this had occurred previously to Huxley and to Gosse, the latter of whom suggested methods of experimentation. Wright's evidence failed to convince Alder who considered the extraneous origin of the nettles improbable. Bergh, too, in a paper, abstracted in the *Microscopical Journal* ('62), described the nematocysts of several additional genera and species and interpreted them as secretions of the cnidophore.

Bergh's failure to discuss Wright's work, led the latter to publish in the next volume of the same journal an abstract of his former paper, together with additional evidence which seemed to confirm the original contention. Grosvenor says: "Owing either to the absence of figures or the aforementioned improbability of the conclusions it contained, this abstract seems to have been overlooked as completely as the original paper." All observers, from 1861 to 1903, failed to acquaint themselves with Wright's work, and assumed that the nematocysts were "manufactured" by the eolids in which they are found. Bergh himself, however, seems afterward to have entertained suspicions.

In the autumn of 1903 G. H. Grosvenor resurrected Wright's results, which, like all other writers, I too had overlooked, and on the basis of new experiments and histological observations gave an excellent account of the origin of the nematocysts. Recently Cuénot ('07), doubting both Grosvenor's statements and mine, re-investigated the subject, and now believes that the nettles are derived from coelenterates. Cuénot's paper, however, contains fewer cytological details than the earlier one by Grosvenor.

EVIDENCE BEARING ON THE ORIGIN OF THE NEMATOCYSTS

One who maintains that the nematocysts of eolids are derived from their coelenterate prey, must be prepared to answer certain questions:

- a. Do eolids feed on coelenterates?
- b. Are the nematocysts of eolids identical with those of their prey?
- c. Are nematocysts indigestible?

d. Can the nematocyst content of an eolid be altered by a change in diet?

e. Does a nematocyst-bearing species ever have individuals devoid of nettles?

f. Can the transfer of nematocysts from coelenterates to the cnidophores of eolids be followed in detail?

The diet of eolids

The literature is full of testimony alleging the close association of eolids and coelenterates. Moreover, eolids have frequently been observed while feeding on their coelenterate neighbors. I have often seen them browsing on *Tubularia crocea*; on *Eudendrium racemosum*; and on actinians. Many species are so translucent that their food gives its color to the entire animal, and individuals which have fed on the pink heads of *Eudendrium*, or the somewhat paler ones of *Tubularia*, have the exact tints characteristic of these hydroids. Careful examination shows that the colors are due to undigested food in the alimentary canal and its diverticula.

Identity of eolidian and coelenterate nettles

As Grosvenor says, "Those who quote the nematocysts of nudibranchs and coelenterates as a striking example of homoplasy or convergence, can scarcely be aware of the astonishing completeness of this assumed convergence." His assertion is supported by a considerable number of cases in which the isomorphism extends to the minutest details. Wright and Cuénot made similar observations. A number of equally striking instances have come to my notice, but I shall describe only two.

Among the sagartias attached in great numbers to oyster shells, at Beaufort, N. C., I found one eolid with nematocysts absolutely like those occurring in the tentacles of the actinian. The undischarged nettle of sagartia is a small rod in whose long axis lies a straight filament. Unexploded capsules answering to this

description were found in the cnidophores of the eolid (fig. 3, *A*). The complete identity of the two kinds was demonstrated by the discharged filaments, for these are characteristically barbed. Near its base (fig. 3, *B*), each thread has a large number of minute barbules directed toward the capsule. This region is followed by one with stouter barbs, and this in turn by one with small. The extreme tip of the thread is bare. Such a filament is sufficiently marked to make its recognition easy.

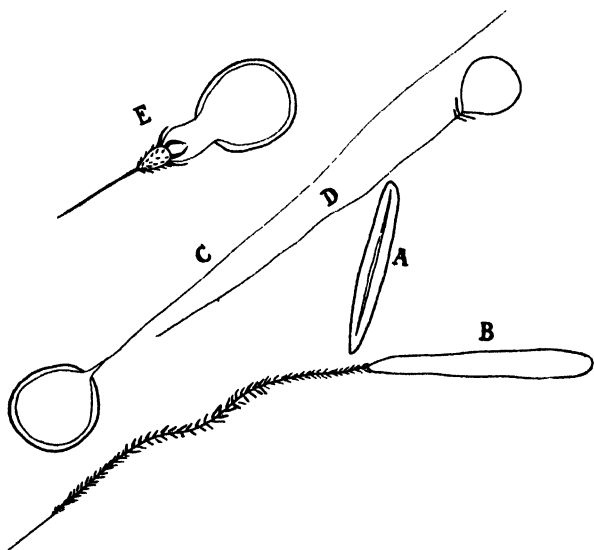


Fig. 3 *A, B*, nematocysts of *Sagartia* and of an eolid which preys on *Sagartia*; *C, D, E*, three types of tubularian nematocysts found in eolids which prey on *Tubularia*.

The most striking case which I have observed is the following: at Beaufort, also, another species of *Eolis* lives on *Tubularia*. An examination of the undischarged nematocysts of this tubularian divulges no great differences among them, but when exploded three kinds are easily recognizable. One (fig. 3, *C*), has a long delicate thread completely devoid of barbules, and widening near the base where it is continuous with an almost spherical capsule. A second type (fig. 3, *D*), less frequent, practically duplicates the first, except in two particulars: the capsule is considerably smaller, and the thread instead of widening at its base, is prob-

vided with two pairs of barbs, of which the stouter is about three times the length of the other. The third type of nematocyst (fig. 3, *E*) is the most common. In this the capsule is ovoid, and the discharged filament presents distinct regions. At the place where evagination occurs there is a bare projection crowned by four stout barbs with points directed toward the capsule. These four barbs originate in a crease between the barren mound and a considerably smaller distal swelling with many minute barbules pointing, like the large ones, toward the capsule. From the tip of this barbulated swelling the undifferentiated portion of the thread arises. The thread itself, however, is not without distinguishing characteristics; it is about twice the thickness of the threads of the first and second type, and about one-fifth as long; it is devoid of barbules and ends in an excessively fine point.

The cnidophores of eolids which prey on *Tubularia* contain nematocysts which in their discharged state agree point for point with one or the other of the three types found in the hydroid. That these three types represent developmental stages of only one kind of nettle seems unlikely, but even if they do, the argument from identity remains unchanged.

Indigestibility of nematocysts

If the source of eolidian nettles is coelenterate food, it follows that the latter is not completely digestible. In a paper on the physiology of nematocysts ('09), I described certain methods of isolation whose success depends on the immunity of the capsules to at least two forms of digestion, peptic and putrefactive. In order to discover whether the nematocysts of eolids could be treated without destruction in the same manner as those of *Hydra*, *Metridium*, and *Physalia*, I subjected a large number of the cerata of *Montagua* to peptic digestion. The experiment gave a positive answer. Without immunity to digestive enzymes eolidian nettles could not be derived from coelenterates, but the fact that they are indigestible is no argument in favor of derivation, since in the opposite case they would probably be equally resistant.

Change of diet

Wright, Grosvenor and Cuénot, have studied the effect of an altered diet on the nematocyst content of eolids, and have demonstrated conclusively that the nettles vary with the food. Grosvenor's results particularly are so striking that there is scarcely need of insisting further on this point. I shall, however, add one case out of a number which I have collected.

In attempting to change the natural food of an eolid, one is confronted with an instance of strikingly specific diet, a condition suggested in nature. Montagua for instance, occurs only on *Tubularia crocea*, even when other hydroids are present. Indeed, late in the summer, when the colonies of *T. crocea* are degenerating or have almost disappeared, while *Eudendrium* is in fair condition, and *Pennaria* is flourishing, Montagua does not change its diet; instead it clings to its food supply as long as this endures, and in the end disappears to return only with the next crop of the hydroid.*

This pronounced specificity interferes with attempts to replace the natural food of a species, but in a few instances I was able to substitute *Aiptasia* for *Tubularia*. The cerata of an eolid treated in this manner contained afterward the nematocysts characteristic of the anemone. A section through such a cerata is copied in fig. 4, and shows in addition to the great number of naturally captured nettles, those artificially introduced (*A-F*).

Eolids devoid of nematocysts

Grosvenor has cited *Calma glaucoides* as an eolid normally devoid of nematocysts. According to Hecht, *C. glaucoides* feeds on the eggs and embryos of *Cotta* and other fish. Whereas this species has neither nettles nor cnidophores, *Calma cavolinii*, the only other member of the genus, feeds on hydroids and has typical cnidophores crowded with nematocysts.

These very suggestive facts can be duplicated within a given species. Thus in an earlier paper ('03), I mentioned an eolid which had been captured accidentally either before or during its

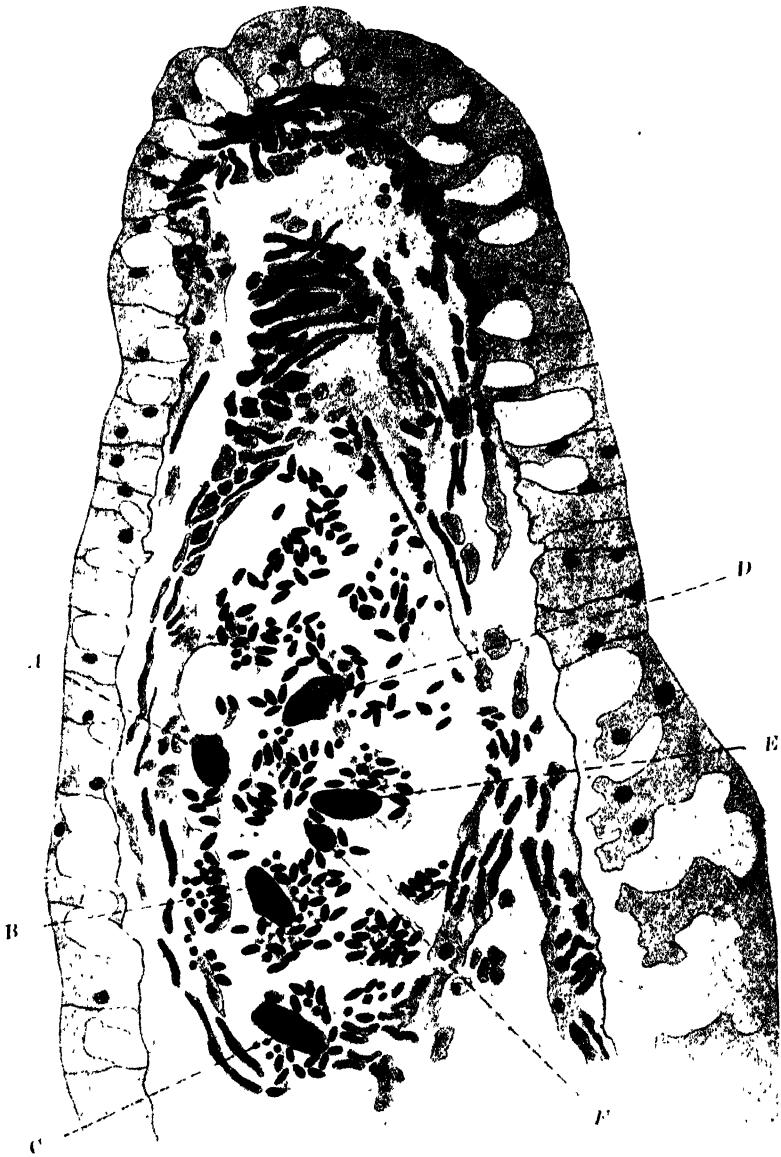


Fig. 4 Longitudinal section through the ceras of an eolid showing the normal nettles in great profusion, and at A, B, C, D, E, F, the artificially introduced nematocysts of *Aiptasia*. Drawn by Mr. Carl Kellner.

metamorphosis, and had completed that process in an aquarium in which it lived for over two months. During the early part of this period, a few hydromedusæ were present, but these soon died out, and the eolid passed the greater portion of its captivity in the absence of coelenterate food. Serial sections, upon careful examination, since repeated, revealed nothing that could be identified as a nematocyst, either in the liver or in the cnidophores.

Even under natural conditions cases of this kind may occur. Like Krembrow, I have found, on several occasions, eolids practically devoid of nematocysts, as well as barren cnidophores among many heavily charged. These facts are easily understood if the nematocysts are derived from coelenterates, but on the contrary view they appear as abnormalities without known explanation.

The experiment of hatching and rearing the young in aquaria free of coelenterates, would give an unequivocal answer could it be carried out. Many difficulties however, present themselves, most important of which are the fatal attacks of the infusorians inevitably introduced with the food and water. Cuénot ('07), nevertheless was able to make tests of a strictly analogous nature. By clipping the cerata and allowing regeneration to occur in the absence of coelenterates, he produced eolids with appendages partly free of nettles. The failure to regenerate cerata entirely devoid of nematocysts was due to the presence of capsules in other portions of the digestive tract, in the lumen of the liver as well as of its diverticula, and engulfed even in the liver cells themselves. Many of these nettles found their way into the regenerating cnidophores, but even so, not in sufficient number to destroy the great contrast between the restored and the amputated appendages. This slight uncertainty in Cuénot's results could be eliminated entirely by starving the animals for four or five days before operating.

The transfer of nematocysts

Cuénot has said that the best evidence of the derivation of solidian nettles is their history. This has been most carefully worked out by Grosvenor, and certain points in it have been

touched upon by Cuénot. In what follows, Grosvenor's account is substantiated in all important respects.

What happens during artificial digestion unquestionably occurs in the alimentary canal of an eolid. The tissues of the food coelenterate are dissolved and the nematocysts remain in great quantities free in the lumen of the tube. Many of them are voided with the faeces, others find their way into the cnidophores.

The passage from the digestive tract proper to the cnidophore is through the ciliated canal. Even were there no direct observa-

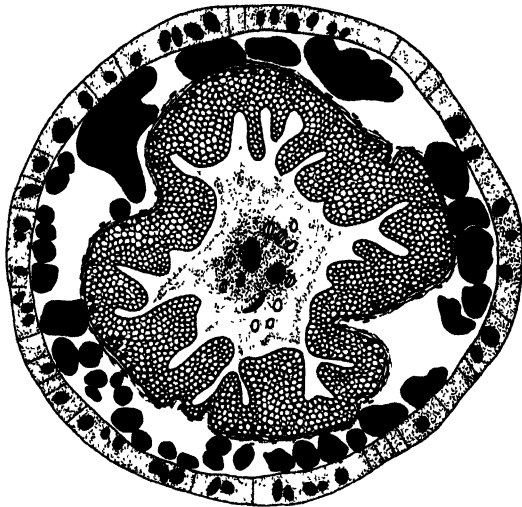


Fig. 5 Transverse section through the base of a ceras showing within the lumen of the liver diverticulum, certain uninterpretable fragments; a considerable number of undischarged nematocysts, and one crescentic diatom.

tions such as those of Hancock and Embleton ('46), Trinchese ('77-'81), and Grosvenor ('03), the occurrence of the same kinds in both cavities which the canal connects would suggest the derivation of the one group of nettles from the other. More suggestive still is the presence of "foreign" bodies in the cnidophore, a fact noted by Hecht, by Hancock and Embleton, and by Grosvenor. I have found uninterpretable fragments of tissue, bodies not tissue-like, and diatoms, in the liver diverticula and in the cnidophores. The ciliated canal apparently has no power to distinguish

other indigestible bodies from nematocysts, but this inability to select is not shared by the cnidophages, for so far as I know, these ingest only nematocysts.

The cnidophages are derived from a zone of "embryonic" cells, located just distally to the ciliated canal. Fig. 6, a drawing of the proximal portion of a longitudinal section which happened not to strike the canal, shows the embryonic zone (*EMB. Z.*). Its cells have either no boundaries or incomplete ones; the nuclei are large and contain coarse chromatin granules; and the cytoplasm is undifferentiated. From this region of proliferation the oldest cells

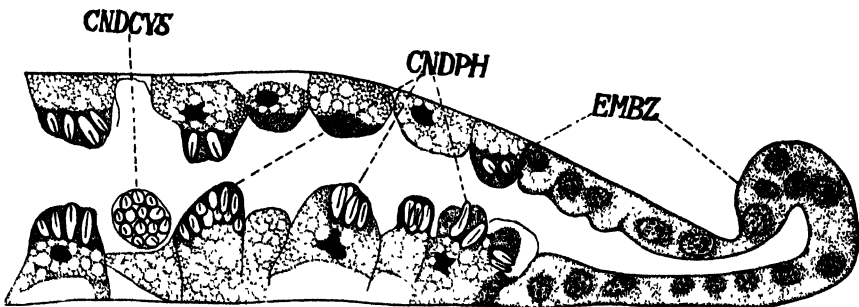


Fig. 6 Median longitudinal section through ceras; *EMBZ.*, embryonic zone; *CNDPH.*, cnidophages; *CNDCYS.*, cnidocysts.

are pushed upward and enter the zone of cnidophages (*CNDPH.*) where, after ingesting a number of nettles inversely variable with the size of the engulfed capsules, they become converted into cnidocysts (*CNDCYS.*).

No single description of the cnidophore would prove satisfactory since the various zones that compose it present phases which depend in part on the age of the appendage, in part on the number of nematocysts ingested. In cerata in which "feeding" is actively going on, or in which it might go on, certain cells show evidence of pseudopodial activity. In the transverse section, fig. 7, the lumen of the cnidophore is traversed and rendered labyrinthine basally, by projections from its bounding cells. Processes from several cells may fuse into larger bridges with, at

times, branch connections to other cells. Though visible only in sections, the projections suggest activity, as if they had been fixed in the act of moving, or flowing. It is interesting that cnidophores well-stocked, do not contain these labyrinths. Apparently if the "fishing" results in a "catch," the cells withdraw their pseudopods.

Not all the cells produced by the embryonic zone become cnidophages. In addition to these, certain interstitial cells are formed



Fig. 7 Transverse section through a ceras in which the lumen is made labyrinthine by pseudopodia that project inwards from the bounding cnidophages. Drawn by Mr. Carl Kellner.

whose fate is quite different. Fig. 8 illustrates part of a longitudinal section through basal cnidophages. The cells have ingested no nematocysts, but those which were about to, differ distinctly from the prospective interstitial cells. The latter are very narrow, have near their bases small densely staining undifferentiated nuclei, and have cytoplasm, which, wherever visible, resembles that found in the embryonic cells. Near the lumen of the cnidophore the interstitial cells have no membranous boundaries, although elsewhere they are definitely delimited.

The prospective cnidophages undergo striking changes which emphasize still more the disparity in size between them and the interstitial cells. The young cnidophage enlarges, partly by the development of great vacuoles in the cytoplasm, (fig. 8), and partly because the cell contents withdraw from all except certain portions of their boundary, remaining connected with it only here and there by strands.

When the differentiation of the cnidophage is complete, it presents the appearance shown in fig. 9. Each cell has a receptive eminence in which the cytoplasm remains naked and in the

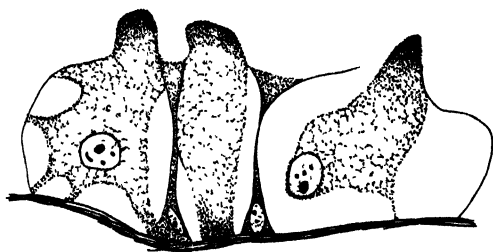


Fig. 8 Showing young cnidophages with blunt pseudopodia. Also interstitial cells.

embryonic condition. From this point pseudopodia may be sent out, but in appendages in which ingestion has taken place, only short, blunt processes are found.

After the cnidophage has ingested its fill of nettles, degenerative metamorphosis sets in. This process is characterized chiefly by retrograde changes in the nucleus, which gradually loses all visible differentiation, and becomes irregularly lobed or stellate (fig. 6). As the finished cnidocyst shows no histological details other than the nematocysts and their enclosing membrane, the inference that nucleus and cytoplasm both degenerate completely seems well founded.

The complete history of the cnidocyst remains in doubt. Grosvenor was not able to determine with certainty where the bounding membrane comes from, nor have my own efforts to

solve the problem met with better success. A suggestion is all that I can offer. The wall of the cyst is tough, and has considerable thickness. It is apparently formed during the period when the cnidophage is degenerating. Under the circumstances it is not unlikely that this cell has nothing whatever to do with the formation of the cyst, but that the membrane in question is traceable to other cells. Grosvenor indeed suggested that the interstitial cells might aid in the secretion of the cyst; to me it seems equally plausible to assume that these cells alone are responsible. The best evidence which supports this view is fig. 10, in which are

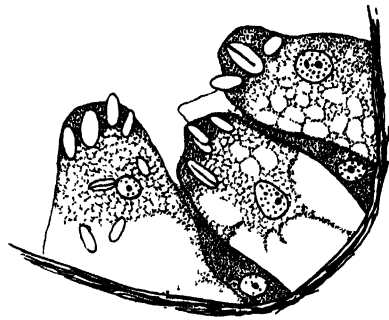


Fig. 9 Ingesting cnidophages showing differentiation and shrinkage from cell membranes. Also interstitial cells.

shown degenerating cnidophages, enclosed within compartments formed by interstitial cells which themselves have lost practically all of their original characteristics. As the figure illustrates they are reduced to mere membranes. Since each nettle-sac is complete, and in the ripe state capable of isolation from its fellows we must suppose on this view of the matter that the interstitial cell which lies between two or even three cnidophages contributes an independent portion to as many cnidocysts.

However they originate, the cnidocysts in their finished form are mere bags, thin-walled and transparent, filled with undischarged nettles. The cysts may lie free in the lumen of the cnidophore, or loosely attached to its walls. In either case they are easily extruded through the cnidophore by contractions of the cnidophoral musculature.

DISCUSSION

While the demonstrated identity of eolidian and coelenterate nettles is clearly not due to homoplasy, the actual occurrences are not so simply disposed of. Instinctively one wonders how such complex interrelationships between two totally distinct sets of organisms could have come about, and to what extent the pro-

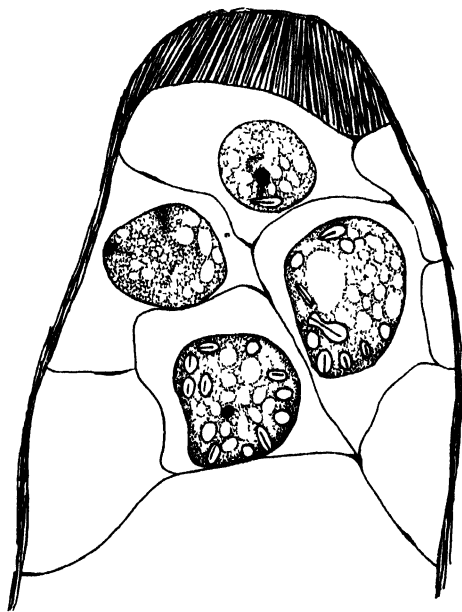


Fig. 10 Tangential section through the cnidophore showing young enidocysts enclosed in spaces limited by degenerating interstitial cells.

cesses described may be said to be adaptive. Before attempting to deal with the questions that arise, it will be well to summarize briefly what can be easily understood if the collected evidence is valid.

If the accounts of Wright, Grosvenor, Cuénot and myself, are to be trusted, it is easy to see why the nematocysts should be found inside of entodermal cells and their derivatives; it is easy

also to understand why, as has been pointed out by Krembrow, the nucleus of the cnidophage takes no part whatever in the "manufacture" of the nematocysts; further the occurrence of individuals and of cerata devoid of nettles is readily explicable; and finally the identity of eolidian and coelenterate nettles is no longer a case of convergence that exceeds the probabilities of homoplastic evolution. However, some things remain to be explained.

The first requisite for the development of these remarkable relations, is that eolids shall be immune to the netting organs of coelenterates. Since nudibranchs are animals of unusual delicacy and apparently without protection, the freedom with which they crawl over hydroids and actinians, and browse upon their heavily charged tentacles, is intelligible only on the assumption that the dangers in the midst of which they live do not apply to them. It is not without interest to inquire how this can be.

The thought that most naturally comes to one is that in the course of time immunity has developed either by the elimination of individuals most prone to succumb to bombardment from nematocysts, or that in each generation the individuals, by being constantly under fire, gradually become indifferent to the punctures and stings, and finally fail entirely to react to stimuli to which the uninitiated respond by lively movements, and possibly by sensations of pain. This reasoning, however, rests on the assumption not only that eolids are vulnerable, but that they are under fire, and both premises require qualification.

That eolids are sensitive to the bombardment of nematocysts can be shown by transferring an animal which normally lives on *Tubularia* to the disc of a large *Aiptasia*. If the animal is not swallowed, it may fall off and reach the bottom of the dish in a perfectly rigid paralytic state. Occasionally spasmodic and angular movements may be observed, but recovery is rare as the animal usually dies despite many precautions.

If the discrepancy in size be reversed, so that the eolid is considerably larger than the *Aiptasia*, the latter seems to affect to a marked degree only the highly sensitive foot-tentacles, and the eolid under certain circumstances may devour the actinian. The attack is made at the base, and as the anemone immediately re-

tracts its disc when assaulted, it is devoured in its least dangerous state. The outcome of the two experiments is thus very different.

It follows from this that even an absolute immunity to the nematocysts of one coelenterate does not insure immunity against those of another. The absolute immunity itself, however, does not exist. It has been said by several writers that eolids showing evidence of having been punctured either from without or within by the sort of nettles in which they normally traffic are unknown. Fig. 11 represents a cross section through a ceras taken from an

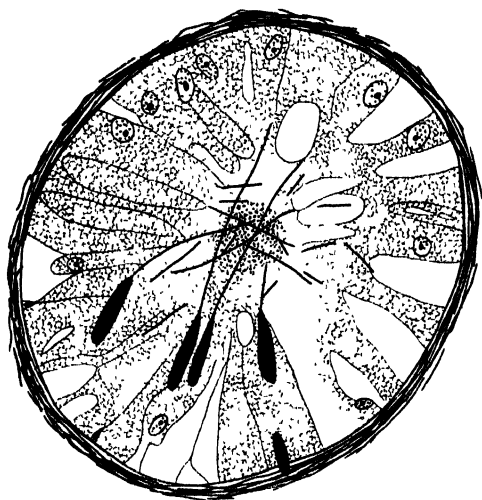


Fig. 11 Cross section through the base of a ceratophore in which intra-ceratal explosions have taken place.

eolid feeding normally on *Aiptasia*. The particular section is by no means representative, and gives a very inadequate idea of the number of discharges which has occurred in this appendage. My reason for choosing it is the ease with which the individual filaments can be traced to their capsules. Other sections contains inextricable snarls of threads.

Immunity, therefore, is relative and depends on several interesting details. It is not unlikely that phyletic as well as individual acclimatization plays a rôle; more important, however, are the reactions of coelenterates to eolids, and most important the nema-

tocysts themselves. When touched and particularly when about to be cropped, the tentacles of a hydroid or of an actinian contract, and consequently fewer nematocysts are likely to discharge than if the tentacle remained expanded. More to the point even than this is the fact that when tentacles are cut off quickly, no discharges, or only few occur. It follows, then, that eolids are not under a heavy fire to begin with.

But such fire as they are under does not of necessity reach them, for, aside from the protection offered by the mucus which they secrete, the bodies of eolids are not the sort to be easily punctured by nettling threads. Certain experiments performed while studying the physiology of nematocysts ('09) show that a soft surface is apt to ward off the filament, and further that penetration is most easily effected when the thread is under its maximum speed at the beginning of the explosion. In order that a surface may be broken in upon, it must be very close to the mouth of the discharging nettle. Furthermore, Toppe ('09) has shown by extremely careful and interesting observations that the most common type of hydroid nematocyst is especially adapted for puncturing hard, chitinous surfaces and not soft indentible ones.

That discharge of the nematocysts after ingestion does not take place, my own observations have shown to be untrue. nevertheless such explosions are rare. In the paper on the physiology of nematocysts ('09) it was shown that the discharge of the filament is really an explosion traceable to a rise in the internal pressure of the capsule. As this rise results normally from osmosis and as the fluids in the digestive tract of eolids are probably more concentrated than those inside the nematocysts, one can easily understand why explosions within the cerata should be rare enough to have hitherto escaped detection.

Finally, it is not unlikely that immunity in the narrower sense renders ineffective such penetrations as may occur. The evidence of a "Reizgift" postulated to explain the irritations set up by nematocysts, does not appear to me as yet quite conclusive ('09). On the other hand, the evidence that there is present in nettles another poison, the hypnotoxin, responsible for the paralysis of punctured animals, seems well grounded. It is quite possible

that there exists in eolids an anti-body capable of neutralizing hypnotoxin, provided this substance is not introduced in too large quantities, or in a variety to which the anti-body is not adapted. If this sort of immunity exists, then the total "immunity" of an eolid to a particular type of nematocyst is due in part to the various factors which prevent the mollusc from being punctured; in part to acclimatization, phyletic or otherwise, to the mechanical effects of such punctures as are made; and finally to the neutralization of the small quantities of hypnotoxin introduced.

With the demonstration that immunity exists, but is relative and dependent on several distinct factors, the way is paved for a consideration of the origin of the nematocyst-storing habit.

The indigestibility of the nettles renders their elimination necessary. One way in which this is accomplished is by avoidance with the faeces; another by avoidance through the cnidopores. Why this second method should have developed is difficult to determine. According to Hecht ('96), *Calma glaucoides* is a typical eolid except in two respects; it has neither cnidophores nor nematocysts. *Calma cavolinii*, the only other member of the genus has cnidophores filled with nettles. As *C. glaucoides* in all probability did not branch off from the common eolid stock before the introduction of nettles, and as its habit of feeding upon the eggs and embryos of shore fish, such as *Cotta*, is, with equal probability secondary, it seems likely that the abandonment of the nematocyst habit is quickly followed by degeneration of the accessory organs of elimination. If this be true, one may infer that the presence of nematocysts influences the production of cnidophores, much as an insect sting may bring on, in a plant, the formation of galls.

I do not know what it is in nematocysts that renders their prompt removal from the digestive tract proper, desirable, nor whether the immediate end, whatever it be, is accomplished by the means employed. That elimination, or at least storage, is somehow useful, while not absolutely proved, is certainly probable, for the idea that it is profitless or harmful is difficult to harmonize with the complexity of the structures and processes involved. It must be borne in mind that a ciliated canal makes

possible the easy passage of nematocysts from the digestive tract to the cnidophores; that specially adapted cnidophages store the nettles, and become converted, with the aid of interstitial cells, into cnidocysts; that these step out of line and are forced by means of a special musculature out of the cnidopore; and that there is a zone of "embryonic" tissue which furnishes a supply of cells for all the purposes of the cnidophore. In addition to elimination there are other ways in which the overloading of the cerata is prevented, for new appendages are constantly forming, and by autotomy, the largest and presumably best stocked cnidophores are frequently cast upon the slightest stimulation. Even in the absence of assignable specific reasons for the voidance of nettles it seems reasonable, in face of these facts to assume that the processes now known to occur are useful, and that elimination of nematocysts is the primary function of the cnidophore proper.

Grosvenor has given a possible history of this function and of the system of organs that carry it on. "In molluscs," he writes, "other than the cladohepatic Nudibranchs, the food is digested in the stomach, where absorption takes place . . . In Tritonia, therefore, the anus suffices for the passage of nematocysts out of the body. But in the Cladohepatica, part of the food is digested in the gastric gland, quite fresh pieces of hydroid being found in the ducts and ceratal diverticula of a recently fed *Æolid*. . . How Dotonids which habitually feed on hydroids and have no aperatures in their cerata, get rid of the nematocysts, I cannot say; perhaps by throwing off their cerata, which as is well known they do with great ease. When an aperture for the extrusion of nematocysts had once been acquired, it would be obviously advantageous that the distal end of the "hepatic diverticulum" should be modified to form a cnidosac where the nematocysts might be stored." With this attempt at phylogenetic explanation, and the reasons cited to support it, I agree with one exception: to me it seems more probable that the "cnidosacs" as well as the habit of storing nettles are both older than the cnidopore from which extrusion takes place, and furthermore, that primitively, eolids probably cast off their excess nettles by autotomizing the cerata.

As a mechanism for voiding nematocysts, the cnidophoral

system is clearly fit. This however, does not tell us why elimination should take place, nor whether either this or the complicated machinery to secure it are more than compromise responses to the biological problem, whatever it be, that lies back of both. As long as the nematocysts were considered part of the organic make-up of eolids, a sufficient reason for their presence and behavior was found in their supposed utility; nor is the case necessarily altered by the demonstration that the nettles are visitors rather than natives in the bodies of these molluscs. There are many details also which seem to add force to the argument from utility. It is a fact that the nematocysts of eolids explode on coming into contact with sea water or a medium more dilute. It is natural to infer that they are neither more nor less effective when extruded from a Montagua, than they would have been had they exploded in the mother tissues of the Tubularia that made them. It is likewise true that the explosion of these borrowed nettles is accompanied by sensations of pain if allowed to take place on a sensitive part of the body, between the fingers or on the tip of the tongue. Moreover eolids behave exactly as though a high degree of utility attached to the nettling organs. When stimulated, thermally or mechanically the nematocysts are extruded, and explode in great numbers. Grosvenor remarks:

"No one can have witnessed the reaction of an Aeolid to various stimuli . . . without being convinced that the cerata are used as a means of defence. The body is contracted, the head being often nearly telescoped into the trunk while the cerata are erected and waved about, especially in the direction of the foreign body, and are often considerably extended.

Under these circumstances Grosvenor nevertheless did not witness the actual extrusion of nematocysts. Cuvénot ('07), in discussing the same subject, writes:

"Lorsqu'on touche un Eolidien avec une baguette de verre, il prend aussitôt une attitude particulière; les papilles s'érigent, s'allongent et se tournent autant que possible vers le point lésé, comme si elles s'orientaient pour cribler l'ennemi de nématocystes; mais en réalité, comme le dit très justement Grosvenor, il y a très peu ou même pas du tout de

nématocystes rejetés au dehors par la contraction des sacs. Cette attitude est donc purement émotive, et n'a pas d'effet défensif direct. Ce n'est que lorsqu'on tracasse violemment l'animal, ou mieux encore lorsque les papilles sont arrachées et comprimées, qu'il sort des sacs une masse de cellules nématophages et de nématocystes, qui explosent aussitôt. Il y a de très bonnes raisons, tirées du mode de fonctionnement des nématocystes, pour croire que cette explosion non dirigée ne peut avoir qu'un effet insignifiant . . . ; mais laissons cela et essayons des expériences."

In combats with its own kind it is not surprising that the dorsal appendages should be attacked, for not only are eolids "immune" to the nematocysts of the cerata, but these on stimulation are erected and project like the quills of a porcupine. The enemy comes unavoidably into contact with them. It is surprising, however, to witness exactly the same thing when a blennie meets an eolid. If the blennie is hungry it behaves in a seemingly pugnacious manner, darts at the eolid, seizes a mouth full of cerata, and pulls and twists them off as though they were tid-bits. As the blennie is one of the commonest denizens of regions inhabited by eolids we have here one instance at least, in which the defensive value of the nematocysts can be discounted.

Many experiments with *Fundulus heteroclitus* were made. In general this fish, in the first trial of an experiment will take an eolid, but almost instantly regurgitates the captive. Contrary to my earlier statement ('06), a second or even third trial may be made, depending on the degree of hunger, and if the *Fundulus* is very hungry, the eolid may finally be swallowed permanently. As the cerata are cast during these successive captures and regurgitations, it might be that the final retention is due to the loss of the nematocysts which at first rendered the eolid disagreeable.

Further experimentation shows that this view is untenable, for if eolids completely devoid of cerata, or forms naturally without nettles are used, a fundulus will repeat all the performances which it goes through when dealing with a normal individual bearing nematocysts. Leaving aside altogether the nettleless nudibranchs, the eolids employed in these experiments were not

so seriously crushed during the trials that cost them their cerata, that the behavior of the fish could be due to nematocysts in other parts of the animal, so that obviously unpalatability to certain fish is a common property of nudibranchs, and by no means limited to the nettle-bearing species or individuals.

If these results, which are in complete harmony with those of Cuénot, are surprising, this is largely due to a common misapprehension as to the nature and significance of nematocysts. Whenever the word nematocyst is employed, it not unnaturally calls to mind the stinging artillery of a *Discomedusa*, or of a Portuguese man-of-war. Such armament is quite misleading, for its tremendous effects are by no means typical. Indeed the forms from which nudibranchs derive their nematocysts are themselves subject to attacks from the same fish which under certain circumstances will devour an eolid, and if the nettles of *Eudendrium* and of *Tubularia* do not protect them against browsing blennies, pinfish, and minnows, it is not to be expected that the same nettles transferred to another animal will gain in effectiveness.

In addition, the observations of Toppe ('09) suggest an important clew. Apparently the structural specialization among nematocysts is accompanied by specialization of function; for while some kinds serve as nettles, others are prehensile organs, and have nothing whatever to do with the infliction of wounds. These facts are very significant, for, aside from correcting a misconception widely spread, as to nematocysts in general, we now know definitely how the various kinds function and how beautifully each is adapted to certain specific ends. The adaptiveness of these structures is not in themselves, but comes about as the result of the habits of the animals producing them. It would be surprising indeed if a nematocyst, adapted to curl round the chitinous hairs of a copepod should be protective when exploding in the mouth of *Fundulus*.

Toppe's results suggest further that defense is not the original function of nematocysts, but that they are primarily organs of prehension for the purpose of entangling prey. From this standpoint it appears that the netting function proper is secondary,

and that in certain highly specialized Cnidaria, nematocysts subserving the secondary function have replaced the more primitive prehensile organs. As eolids feed on coelenterates provided with large numbers of prehensile nematocysts, the observed inadequacy of the protection afforded is neither more nor less than one might expect.

On the basis of experiments similar to those I have presented in brief, Cuénot has concluded that the defensive value of eolidian nematocysts is slight. This inference, which the behavior of various fish makes valid receives additional support from the observations of Toppe and the conclusions to be drawn from them. It would be a mistake however to postulate complete inadequacy, for unquestionably the presence of nematocysts of all kinds in the cnidophores gives the cerata certain gustatorial peculiarities, which otherwise they would lack. When crushed on the tip of the tongue the sensation produced by a ceras is not unlike that of Tabasco Sauce. If this is added to the peculiarity, whatever it be, that renders nudibranchs in general distasteful to fish, we may conclude that a slight degree of defensive value attaches to the borrowed nettles.

According to Garstang's view ('88), shared by Grosvenor, the bright colors of the cerata serve "to direct the experimental attacks of young and inexperienced enemies to the non-vital papillæ, and away from the vital and inconspicuously colored parts of the body." The habit of responding to stimulation by erection, insures that the cerata shall bear the brunt of any attack whatever the experience of the enemy. As autotomy takes place with the greatest ease, it often happens during an encounter, that an eolid is obscured by a cloud of unattached brightly colored appendages, which, since their owner resembles the background, are certain to catch the eye of the enemy. Granted that the cerata are at least as distasteful as the body of the eolid; that in addition the presence of certain nematocysts adds even slightly to the disagreeable qualities, and one has in hand perhaps one of the minor factors in the persistence of the nematocyst-habit.

SUMMARY

The nematocysts of eolids are derived from coelenterates, and are not to be looked upon as an instance of homoplastic evolution. Their defensive value is slight, partly because nudibranchs are distasteful to their enemies even in the absence of nettles, and partly because many of the ingested nematocysts are adapted to meet exigencies in the lives of coelenterates which do not arise in those of eolids. A reason other than utility must therefore be discovered in order to explain the origin of the nematocyst-storing habit.

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MITOSIS IN *ÆDOGONIUM*¹

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EIGHTEEN FIGURES

A great deal has been written about cell division in *Ædogonium*. By far the larger portion of the literature, however, deals almost exclusively with the interesting and unique processes involving the cell wall; while the nuclear phenomena appear to have received but little attention, only three investigators having recorded and figured noteworthy observations upon nuclear division.

The first of these was Strasburger ('80), who gave a brief account of the division of the nucleus as investigated by him with the aid of the technique in vogue in the earlier days of cytology. The object of the masterly work cited was, as is well known, to point out the fundamental unity which underlies the process of indirect nuclear division throughout the organic world, interpreting this process in the light of that which had been previously observed in the tissues of the higher plants and animals; in consequence, stress was chiefly laid, in the case of *Ædogonium* and other lower forms, upon features similar to those already familiar to students of the higher forms.

The second writer upon the subject was Klebahn ('92). His account was much briefer and consisted largely of a review of the work of Strasburger and the statement of several differences in detail; followed by an effort to find in the division of the nucleus of some cells of the filament something analogous to a reduction or "maturation" division.

The third and last communication is a long and important paper by Van Wisselingh ('08), devoted entirely to an account of

¹ Contribution from the Biological Laboratory, University of Virginia.

karyokinesis in this genus. By the use of an original and valuable (but exceedingly difficult) technique this investigator was enabled to make important additions to and corrections of the results of his predecessors: among the most noteworthy of these is the determination of the number (19) of chromosomes in the species studied by him.

The present paper is an attempt to give an independent account of the process as observed by me; presenting some facts that the writers mentioned have not recorded or figured, and calling special attention to some noteworthy features which are indicated in the drawings hitherto published, but of which only casual mention, if any, has been made.

A species of *Cedogonium* established itself a few years ago in the basin of a public drinking fountain in one of the streets of Charlottesville. When found it was growing abundantly, along with other freshwater algæ; collections were made at frequent intervals for over a year; but although the current through the basin was but slow, it was sufficient to prevent fruiting; and efforts to cause the plant to fruit in still water in the laboratory were unsuccessful. I am, therefore, unable to name the species with certainty. Material from this source was collected and put (while at the fountain) in various fixing solutions: chromacetic (of different strengths), acetosublimite, and chromosmacetic solutions: were used, but by far the larger portion of the material was fixed with chromacetoformol. While this solution causes some shrinkage of the cytoplasm from the cell wall, it is in other respects an admirable fixing agent, and its results have been carefully checked with those of others. While various stains were used reliance was chiefly placed upon iron-hæmatoxylin, with or without a secondary stain.

Frequent attempts were made to study the living cells, but with no satisfactory results. As Van Wisselingh has already pointed out, the extent and density of the chloroplast and the abundance of large pyrenoids render this mode of study well nigh hopeless. While some study was made of celloidin sections, all of the drawings were made from material stained with iron-hæmatoxylin and mounted in dammar.

I wish to acknowledge my obligations to my colleague and former student, Dr. Wm. A. Kepner, who has kindly made the drawings with which this paper is illustrated; he has also gone over the material with me step by step and confirmed or checked my observations. While I alone am to be held responsible for any statement or conclusions presented, I am indebted to Dr. Kepner for many observations and suggestions.

The nucleus of the species studied, when in the resting phase, is quite uniform in size and shape, however much the dimensions of the containing cells may vary. It is almost always approximately spherical (fig. 1), its outline being apparently circular, whether seen as there figured or in a position at right angles to this as in fig. 2, or in a transverse section; occasionally there is some slight distortion, as in fig. 2, but in such case there is no constancy in the direction of the enlargement. Of the hundreds of resting nuclei which I have examined (both in living and in preserved material), I have not found one which could properly be called an oblate spheroid. The diameter is approximately twelve micra, rarely more than thirteen. There is a single large and well defined nucleolus. The chromatin is present in the form of numerous distinct granules of varying size.

The process of mitosis goes on concurrently with the peculiar formation and modification of the "ring" which precedes the elongation of the cell wall. Wherever a ring can be detected upon a cell the nucleus will be found to be in one or another mitotic phase; and such phases are never found until the formation of the ring has begun. These facts are of great service in searching for nuclei undergoing division, either in living or in preserved material.

The first change (fig. 3) is a marked enlargement of the nucleus with a distinct elongation parallel to the axis of the cell: the nucleolus is still conspicuous, and the chromatin is still distributed in granules. This is followed by still greater elongation and the assumption (fig. 4) of a characteristic fusiform shape: the nucleolus is paler; and traces now appear of a reticular arrangement of the chromatin, nothing of which was discovered at an earlier stage.

The reticulum, which is never coarse or conspicuous, speedily passes over (fig. 5) into a well-defined spireme composed of a rather slender and greatly contorted filament. Neither at this nor at any previous stage could a definite "polarity" be recognized. The nucleolus becomes still fainter, though not materially diminished in size. The spireme, which usually lies chiefly near the surface of the nucleus, soon breaks up into a large number of chromosomes, of varying size and great diversity of form (as Van Wisselingh has also shown), which are scattered irregularly throughout the nucleus (fig. 6). Quite a number of nuclei were found in which the spireme was more centrally situated, and others, apparently corresponding, in which the chromosomes were more or less centrally grouped. Fig. 7 is a well marked case of the latter. This centralizing of the spireme and of the newly formed chromosomes is possibly an artifact; but there was nothing in the appearance of the nuclei or of the cells in which it was observed to indicate that such is the case. By the time that the chromosomes are formed the nucleolus is very much reduced in size. It soon disappears altogether.

By this time the nucleus has attained a magnitude and a shape which, while it undergoes some farther modification later in both respects, may conveniently be discussed here. While it is generally true that the nucleus undergoes some increase in size during the early stages of mitosis, the change in this respect in the nuclei of the vascular plants is insignificant when compared with that which takes place in *Cedogonium*. Any estimate of the latter must of course be only approximate. In making such an estimate a nucleus was chosen which was just approaching the metaphase, and whose form was that of a well-proportioned circular spindle: its length was a little over 32μ ; its transverse diameter was 16μ . All the resting nuclei in the same fragment of the filament were approximately spherical in form, with an average diameter of 12μ . The computed volumes of such a spindle and such a sphere are to each other approximately as 4.4 to 1 (corresponding closely to that of two spheres whose diameters are to each other as 20 to 12): the superficial areas of the spindle and the sphere in question are to each other approximately as $2\frac{1}{2}$ to 1.

The change in form is no less noteworthy. As has already been stated, the spherical resting nucleus assumes a fusiform shape early in the prophase (fig. 4); as the metaphase is approached, this is frequently (but not always) modified (fig. 7) by the prolongation of the apices into acute tips. At this time the nucleus is quite plastic, and while symmetrical figures are not uncommon, in many cases the form is more or less modified (figs. 8-12) chiefly by the pressure of adjacent pyrenoids. As is well known, the contour of the nucleus, in the higher plants, disappears in the prophase concurrently with the formation of the achromatic figure: in *Ædogonium* it persists, as will be noted more fully later, till late in the anaphase; it becomes still more elongated (fig. 15), but with little increase in volume.

These changes in size and form are clearly indicated in the drawings of the three observers whom I have cited, but with little or no attention on their part. They appear to me to be noteworthy.

The movement of the chromosomes toward the equator of the nucleus is characterized by great irregularity (figs. 8 and 9), some frequently lagging far behind the others. About this time the first traces of an achromatic figure can be clearly seen, in the form of a weakly developed spindle, which is entirely intranuclear. In many cases it is most strongly marked nearest the equator, giving the appearance of a centrifugal formation. The centrosome-like point shown in fig. 9 is the upturned end of an aberrant chromosome.

As the chromosomes reach the equator they divide longitudinally (figs. 10 and 11) and the daughter chromosomes at once begin to separate. There is the same tendency to irregularity in their movements as in the approach of the chromosomes: the straggling metaphase of this form, as compared with that of most higher plants, having much the character of an attempt to fire a volley on the part of a company of ill-trained militia, as compared with the work of regular soldiers.

Cleavage of all the chromosomes being finally accomplished, the groups of daughter chromosomes move with something of the same irregularity toward the poles of the nucleus (figs. 12 to 15). When they have become clearly separated, the achromatic figure

is represented by a conical group of fibers at each pole (fig. 13); but the most careful searching of a great many nuclei has failed to show connecting fibers between the daughter groups, the region between the groups being occupied by the faintly granular substance of the nucleus, in which traces of linear arrangement of the granules (fig. 15) could at most be seen. Failure to discern the connecting fibers so clearly figured by Van Wisselingh may, of course, be due to an imperfect technique.

During late prophase, but more frequently in early anaphase, what appear to be short fragments detached from the ends of the chromosomes are often seen. These were at first regarded as artifacts, but the examination of a large number of nuclei showed that they were naturally detached masses of chromatin which gradually assumed the form of independent rounded masses of varying size (figs. 11-15).

As the daughter chromosomes approach the poles of the nucleus the latter becomes still more elongated (fig. 15), but with little if any increase in volume: the nuclear contour, even at this late anaphase, is as sharply defined as in the earlier stages of mitosis. Shortly after the individual chromosomes reach the pole they begin to assume a moniliform appearance, with an incurving of their proximal ends, entering upon the telophase. At this juncture there is a sudden disappearance (fig. 16) of the nuclear contour ("dissolution of the nuclear membrane"), and there remains between the newly forming daughter nuclei an ill-defined, but distinct, faintly granular residual mass. This persistence of a definite boundary to the nucleus almost to the close of the mitosis is plainly indicated in the drawings of each of the writers cited, but neither of them has directed to it the attention which in my judgment it deserves.

The daughter nuclei are now separated by a considerable distance. Their rounding off is evidently followed by a sudden and rapid movement toward each other; the next phase is one in which they are closely approximated, while between them (fig. 17) may be seen a flattened granular mass, evidently the compressed residuum of the original nucleus. That their approach is exceedingly rapid is evidenced by the fact that careful searching of a

large number of preparations fails to show any evidence of a condition intermediate to those represented in figs. 16 and 17. The stages represented in these two figures were recognized, and their significance noted by Strasburger (*loc. cit.*, figs. 57 and 59).

The daughter nuclei are now definitely rounded off, and the nuclear contour reappears: from one to three nucleoli may now be seen (later always uniting into one); the moniliform chromosomes have begun to pass over into a reticular structure which later becomes resolved into the discrete granules of chromatin characteristic of the resting nucleus; the mitosis is thus completed. Concurrently with these latest changes the transverse wall appears in the plate of cytoplasm which now extends across the middle of the cell.

The daughter nuclei at first lie close to the newly formed transverse wall; very shortly after they begin to migrate toward the future centers of their respective cells—a fact that was noted by Strasburger. The rate of movement does not appear to be the same in both cases, that in the more distally situated daughter cell apparently being the more active of the two; instances were not unfrequently found in which this nucleus had passed into the region of the ring before that structure had opened but a short distance, and while the companion nucleus was still in close proximity to the transverse wall. The migration of the daughter nuclei was noted and figured by Strasburger.

SUMMARY

Mitosis in *Ædogonium* resembles that seen in the higher plants in its essential features: the chromatin content of the nucleus becomes aggregated to form a filament; this breaks into segments (chromosomes) which pass to the equator of the nucleus, there dividing longitudinally; the daughter chromosomes thus formed pass to the poles of the nucleus and there unite to form the daughter nuclei.

It differs from that ordinarily seen in the higher plants in the following features and their combination.

1. The marked increase in size of the dividing nucleus; from four to four and a half times the volume of the resting nucleus.
2. The conspicuous change in form of the enlarged nucleus, from a nearly spherical to an elongated fusiform shape.
3. The persistence of the definite contour of the nucleus up to the last of the anaphase.
4. The marked irregularity in shape and particularly in size of the chromosomes.
5. The straggling and irregular manner in which the chromosomes approach and recede from the equator, and in the occasional fragmentation of the daughter chromosomes.
6. The intranuclear position of the achromatic figure; its feeble development; its imperfect polarity; its apparent origin in the equatorial region toward the close of the prophase, with subsequent development of fibers toward the poles in the anaphase and the doubtful presence of well defined connecting fibers between the two groups of daughter chromosomes.
7. The sudden coming together of the daughter nuclei during the telophase, compressing between them the irregular residuum of the original nucleus; and in the subsequent migration of one of them to the region of the ring, while the other passes (apparently somewhat less rapidly) to a position near the center of the original cell.

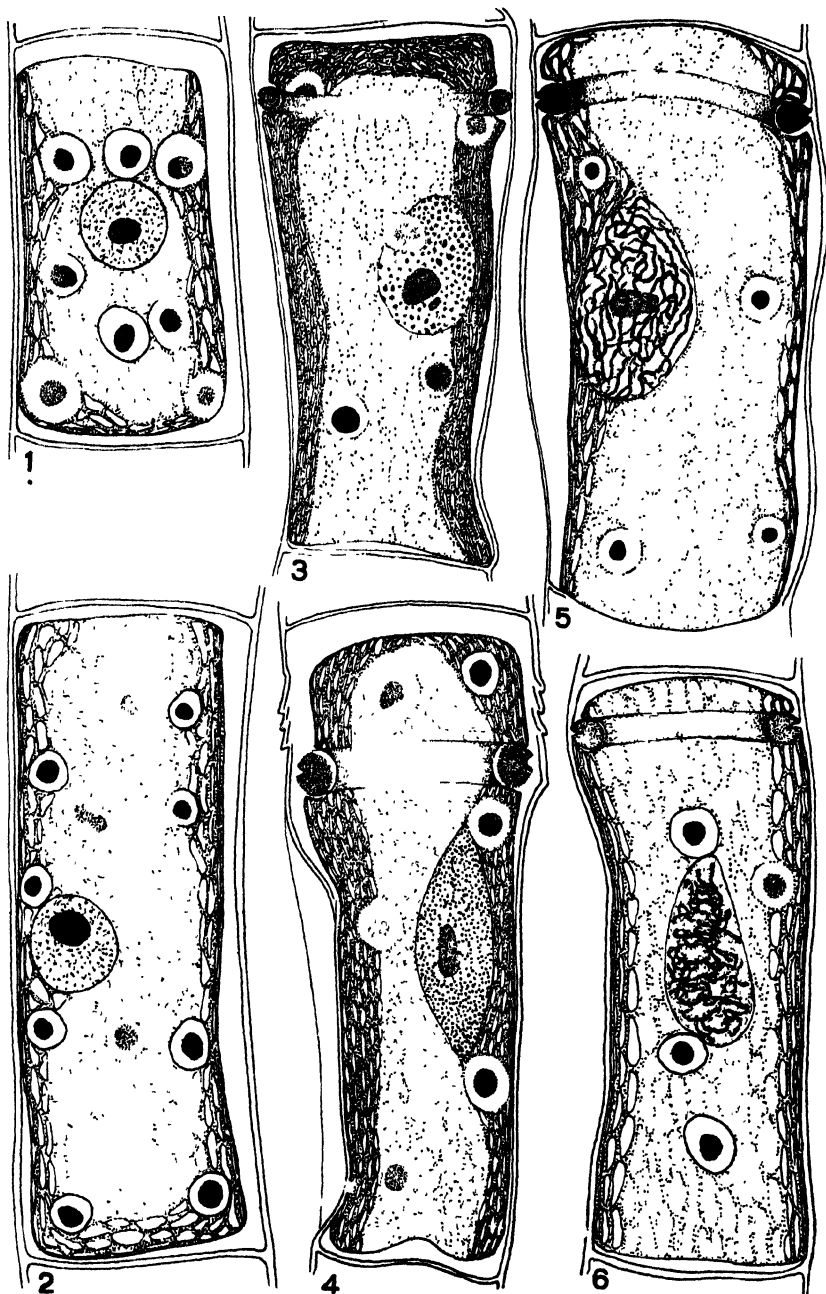
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EXPLANATION OF FIGURES

All figures are drawn with a magnification of 1000 diameters by means of the camera lucida: Zeiss apochromat 1.5 mm., N. A. 1.30; compensating ocular 6.

- 1 "Frontal" view of a resting nucleus.
- 2 "Profile" of a resting nucleus somewhat larger and more irregular in shape than the average.
- 3 Nucleus enlarging and elongating, along with the appearance of the parietal ring.
- 4 Assumption of the typical fusiform shape, with a tendency toward a reticular arrangement of the chromatin granules.
- 5 Well developed spireme.
- 6 Breaking of the spireme into chromosomes.



EXPLANATION OF FIGURES

7 A stage not infrequently observed, in which the newly formed chromosomes are massed at the center of the nucleus : the nucleolus is still present.

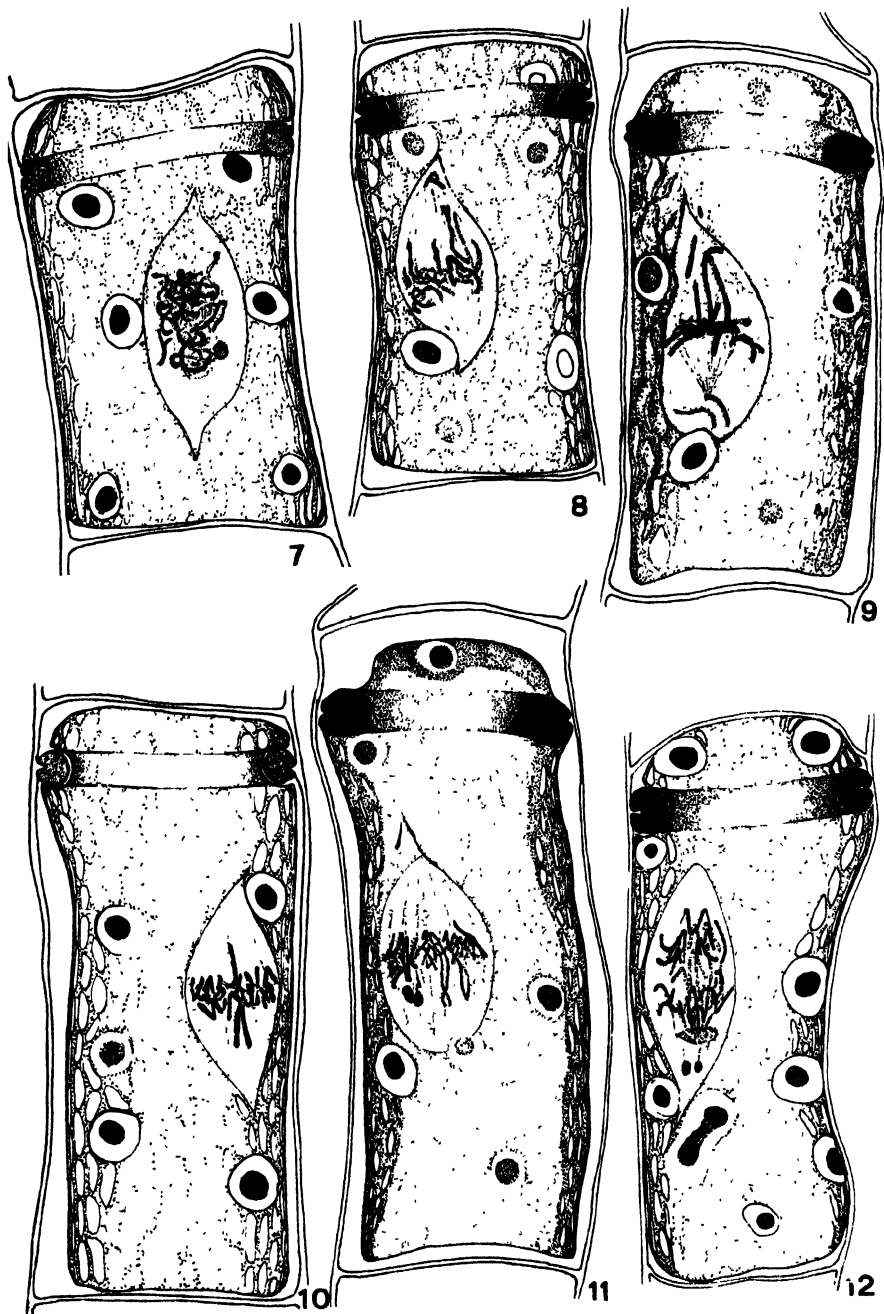
8 Gathering of the chromosomes at the equator with appearance of spindle fibers. In this figure, and those immediately following, only a portion of the chromosomes are represented, for the sake of greater clearness. The nucleolus has disappeared.

9 Another and more frequent mode of assemblage of the chromosomes, showing the marked tendency to straggle. The spindle fibers are well developed at one pole only. The upturned end of a straggling chromosome might at the first glance be mistaken for a centrosome.

10 Separation of the daughter chromosomes.

11 Separation of the daughter chromosomes. The rounded masses shown in this and the following figures represent fragments that have become permanently detached from some of the chromosomes, and gradually assume the form shown. The loose appearance of the spindle is characteristic.

12 Anaphase. The shaded body shown in the lower portion is a pyrenoid seen through the nucleus.



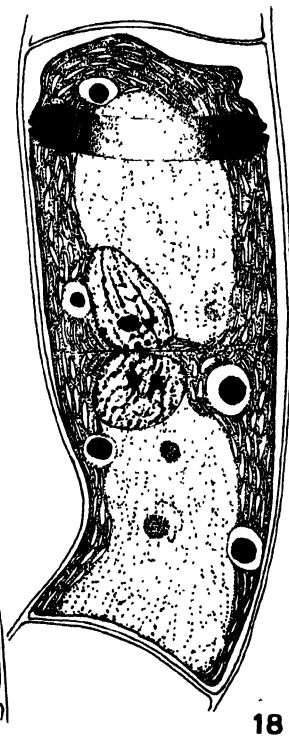
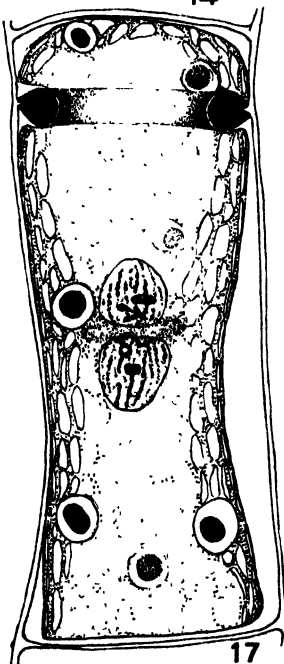
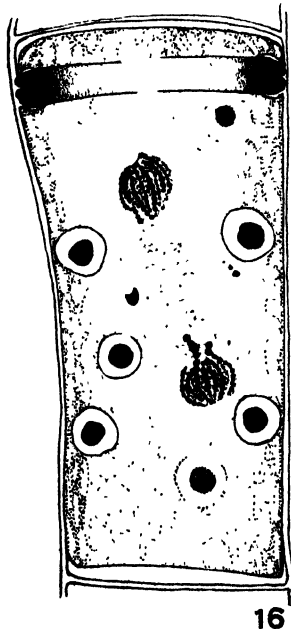
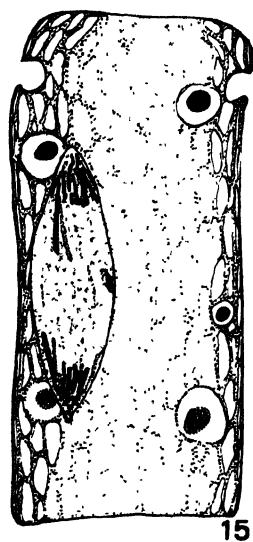
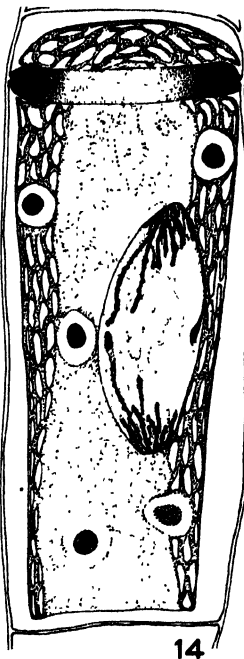
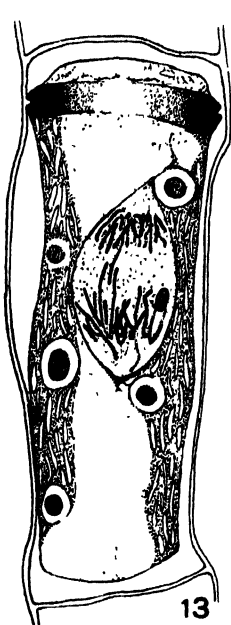
EXPLANATION OF FIGURES

13, 14 and 15 Later anaphases. The tendency of the daughter chromosomes to straggle toward the poles of the elongated nucleus is shown. In 15 the nucleus is at its greatest elongation, and the nuclear contour is still sharply defined.

16 Telophase. The nuclear contour has disappeared; the remains of the nuclear substance are represented by an elongated mass of faintly staining granules.

17 Approach of the daughter nuclei, with reappearance of nucleoli. The nuclear residuum is still present as a flattened granular mass.

18 Division completed, with the appearance of the transverse wall.



THE GENUS ARACHNACTIS

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FIVE FIGURES

In 1846 M. Sars instituted the genus *Arachnactis* for a free-swimming Actinian which he found in the autumn and winter at Florøe Island, off the coast of Norway, and which he named *Arachnactis albida*. Since that time the species has been frequently taken in the waters to the north of the British Isles, and has been especially studied by Boveri (1890), Vanhoffen (1895), Fowler (1897) and E. van Beneden (1898).

In addition to this type species, three others have been assigned to the genus. In 1862 A. Agassiz captured, at Nahant, a form which resembled that described by Sars in many respects, but at the same time showed sufficient differences to warrant its being regarded as a distinct species. Agassiz named it *A. brachiolata*; it has since been taken at Newport, at Wood's Hole, and at Casco Bay, on the Maine coast (Kingsley, 1904).

A third species was first described by McIntosh (1890) from a single example captured in the Bay of St. Andrews, Scotland. It seems probable that this is the same species as the *A. albida* recorded by Bourne (1890) as occurring off the south-west coast of Ireland, and stated also to be not uncommon at Plymouth. At least, this is the opinion of Fowler, who subsequently studied examples from Plymouth and who bestowed on the species the name *A. bournei*. The same form was also studied by Van Beneden (1891).

Finally, among the pelagic Actinians obtained by the "Siboga" during her voyage in the Malayan Archipelago, I have found a fourth species for which I propose the name *A. sibogæ*.

From their general appearance these four species may be divided into two groups, one consisting of *A. albida* and *A. sibogæ*, and the other including the two remaining forms, the most noticeable difference being the greater proportionate length of the tentacles in the members of the first group. In the species that form it these organs are many times the diameter of the disk in length, while in *A. brachiolata* and *A. bournei* they are about the same length as the diameter of the disk or but little longer. A difference of more importance is, however, revealed by an examination of the succession shown in the development of the tentacles.

It is a well known characteristic of the Cerianthæ that their mesenteries appear in couples, the members of each couple lying one on each side of the median sagittal plane of the body. Consequently the intermesenterial chambers will be also arranged in the same manner, except that there will be a single chamber at the ventral edge of the sagittal plane and another at its dorsal edge. The tentacles, both marginal and labial, are outgrowths of the roofs of the intermesenterial chambers, and will therefore also be arranged in couples, except that there will be a median ventral tentacle corresponding to the median ventral chamber. No tentacle develops in the roof of the dorsal median chamber, and hence the number of tentacles of the marginal series at least, will be odd in all later stages of development. It has been found, however, that the median ventral tentacle in both the marginal and labial series, makes its appearance only after a number of couples have formed—indeed, it may altogether fail to develop in the labial series—and the number of couples that precede it in development appears to be definite for a given species.

The marginal tentacles need alone be considered here. Boveri (1890) found that in *A. albida* there was no trace of a median marginal tentacle in larvæ which already possessed three couples of well developed tentacles of the marginal series as well as the two tentacles of the fourth couple in process of development, and Van Beneden (1898) found that in larvæ of the same species in which there were three couples of well developed tentacles and a fourth couple whose members were somewhat smaller, there was present a still smaller marginal tentacle (fig. 1, Tm). Hence it may be

concluded that the median marginal tentacle of *A. albida* does not make its appearance until after the fourth couple is recognizable.

In the youngest example of *A. sibogæ* that I had for study (fig. 2) there were four couples of marginal tentacles, each many times the diameter of the disk in length, and in addition a single small tentacle, little more than a tubercle, situated on one side of the median line and dorsal to the member of the fourth couple of that side. This last small tentacle is evidently a member of the fifth couple of tentacles, its fellow not yet having made its appearance, since it is a rule in the *Ceriantheæ* that the members

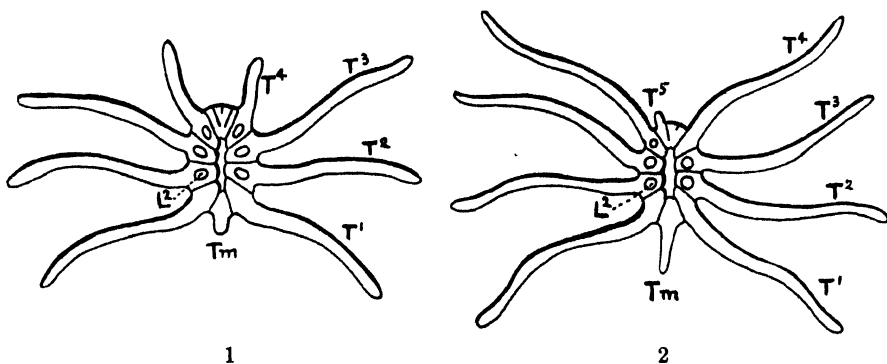


FIG. 1 Diagram of an oral view of *A. albida* at the time of the formation of the median marginal tentacle. L² = labial tentacle of second couple; T_m = median marginal tentacle; T¹ - ⁴ = first, second, third and fourth couples of marginal tentacles (adapted from E. van Beneden).

FIG. 2 Diagram of an oral view of *A. sibogæ* at the time of appearance of the median marginal tentacle.

of one side of the body are always a little in advance of those of the other side in development. The median marginal tentacle was also present and was much smaller than the members of the first four couples, but decidedly larger than the representative of the fifth couple, its length being about equal to half the diameter of the disk. It would seem from this that in this species, as in *A. albida*, the median marginal tentacle makes its appearance only after the formation of the fourth couple and before that of the fifth.

In *A. bournei* and *A. brachiolata* the conditions are different. Of the former species, Van Beneden (1891) observed a stage in which there were two couples of well developed marginal tentacles and a third couple was represented by two very much smaller tentacles, which were themselves decidedly unequal in development, one being only about half the size of the other (fig. 3). A median marginal tentacle was also present, being of about the same size as the smaller member of the third couple, and from this relation, combined with the fact that in younger larvæ possessing only two couples of tentacles there was no trace of the median ventral tentacle, Van Beneden concludes that in this species the median tentacle develops synchronously with the members of the third couple.

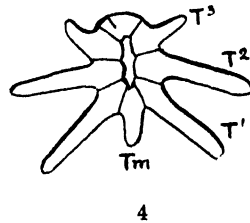
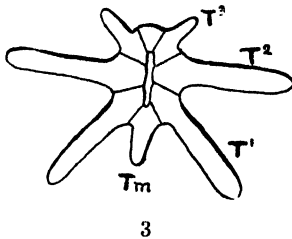


FIG. 3 Diagram of an oral view of *A. bournei* at the time of the formation of the median marginal tentacle (adapted from E. van Beneden).

FIG. 4 Diagram of an oral view of *A. brachiolata* at the time of the formation of the median marginal tentacle.

In *A. brachiolata* I found (1891) practically the same succession. In larvæ with three couples of mesenteries there were only two couples of marginal tentacles, the third couple being represented however, by merely tuberculiform elevations of the disk. In older forms, in which the fifth couple of mesenteries was in process of development, the median marginal tentacle had appeared and had reached a stage of development in which it was practically the same size as the members of the third couple, which, on their part, were much smaller than the membrane of the second and first couples (fig. 4). It is evident, therefore, that the median ventral marginal tentacle of *A. brachiolata*, if it does not appear syn-

chronously with the members of the third cycle, at least succeeds them at a very short interval and undergoes a practically synchronous development.

To state the difference in the two groups concisely, the median marginal tentacle in *A. albida* and *A. sibogæ* develops only *after the fourth couple* of tentacles have appeared, while in *A. brachiolata* and *A. bournei* it develops practically *synchronously with the third couple*.

The difference in the two groups may also be shown by a comparison of the time of development of the median marginal tentacle with that of the labials. In all Ceriantheæ in which their development has been observed the labial tentacles lag behind the marginals, and this is especially noticeable in the cases of the median ventral labial and the first couple, which do not make their appearance until several other couples of labials have developed. In neither *A. brachiolata* nor *A. bournei* is there any trace of labial tentacles at the time when the median marginal makes its appearance, but in the other two species the case is different. In the youngest individual of *A. albida* studied by Boveri (1890) the fourth couple of marginal tentacles was in process of development and there were present two couples of tuberculiform labials, corresponding to the second and third couples of marginals. In the youngest example studied by Van Beneden (1898), in which the median marginal was present, though quite small, there were three couples of labials, corresponding to the second, third, and fourth marginal couples, those corresponding to the fourth marginals being noticeably smaller than the others. Hence it may be concluded that in this species the median marginal tentacle develops contemporaneously with or just before the fourth¹ couple of labials.

In the youngest larva of *A. sibogæ* two couples of labial tentacles are present, namely the second and third, and a single member of the fourth couple also occurs as a small tubercle. It has already

¹ This enumeration is based upon the order of the interspace to which the tentacle belongs, counting from the mid-ventral line, and not on the order of their succession. Thus, the third couple to develop is the fourth couple in the final arrangement.

been pointed out that in this same larva there was a single representative of the fifth marginal couple and that the median marginal judging from its size, is earlier in its development. It seems probable that the fifth marginal and fourth labial couples develop simultaneously and therefore the median marginal probably precedes in its development the fourth couple of labials.

There is essential agreement, therefore, in this respect between *A. sibogæ* and *A. albida* and a decided difference from what obtains in the other two species. Unfortunately nothing is at present known as to the relative times of development of the labial couples as compared with the marginals in *A. brachiolata* and *A. bournei*, nor is it known whether any of the labial couples appear before the third marginals in *A. albida* and *A. sibogæ*. The two labial couples in Boveri's youngest *albida* larva may both have appeared subsequently to the development of the third marginal couple, and if this be the case there may be an identity in the order of appearance of the labials as compared with the marginals in all four species. But whether this prove to be so or not, there is a striking difference in the development of the labials at the time when the median marginal develops.

Turning now to the arrangement of the mesenteries I wish to call attention to a peculiarity which occurs in *A. albida* and *A. sibogæ* and has not hitherto received the recognition that it deserves. Unfortunately it is not possible at present to definitely assert its absence in the other two species.

The ventral couple of mesenteries in all *Ceriantheæ* is associated with the single siphonoglyph and the mesenteries composing it are in all cases short, sterile and destitute of mesenterial filaments. The members of the succeeding couple, on the other hand in the majority of species at present known, are very long, extending to the aboral extremity of the body, and are fertile and provided with mesenterial filaments. These mesenteries have been termed the continuous mesenteries, an expression which may conveniently be replaced by telocnemes. In 1904, however, Roule described a form which departed from this arrangement, establishing for it the genus *Pachycerianthus*. In this the telocnemes are the fourth couple and not the second, and I have found the same arrangement

in two species of the Siboga collection, as has been in part already noted elsewhere, and Cerfontaine (1909) also has recently described it as occurring in *C. oligopodus*. The discovery of this new arrangement of the primary mesenteries is a very important contribution to our knowledge of the Ceriantheæ, and makes necessary a revision of the classification of the group. For the arrangement of the mesenteries does not stand alone, but is apparently associated with another striking structural peculiarity.

In his report on the Actinian larvæ obtained by the Hensen Plankton Expedition, Van Beneden (1898) drew attention to the occurrence of what he termed acontia in a number of the larvæ.

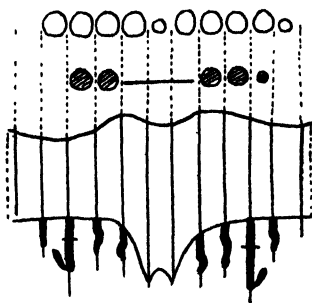


FIG. 5 Diagram of the arrangement of the mesenteries of *A. sibogæ*.

These structures resembled the Hexactinian acontia in being filaments attached at one extremity to certain mesenteries just below the lower end of the mesenterial filament. They are, however, very much shorter than the Hexactinian acontia and are limited to certain mesenteries. The point which they have of interest in the present connection is that in some forms they occur upon the mesenteries of the second couple (counting from the mid-ventral line), while in others the most ventral acontia occur upon the mesenteries of the fourth couple. This latter condition is the one which obtains in *Arachnatis albida*, according to Van Beneden's account of that species, and is clearly shown in Boveri's Plate XXI, fig. 3. Furthermore, it is the condition that I have found in the older examples of *A. sibogæ* (fig. 5). But in these

same two species, at the stage in which the acontia occur, the mesenteries of the fourth couple are longer than any of the others that are present, and the same relations may be seen in other forms described by Van Beneden, as for instance, in species of the genera *Dactylactis* and *Ovactis*, and in forms occurring in the Siboga collection. On the other hand, in those forms in which the mesenteries of the second couple are the most ventral ones provided with acontia, as in the genus *Apiactis* as described by Van Beneden and as I have found in the genus *Peponactis*, it is these same mesenteries that are the longest.

Bearing in mind the fact that in the genus *Pachycerianthus* it is the fourth couple of mesenteries that become the telocnemes, it seems fairly certain that in those larvæ in which the mesenteries of the fourth couple are the longest and at the same time the most ventral acontiferous ones, these mesenteries become the telocnemes. Or to make the statement more general, it is probable that the most ventral acontiferous mesenteries become the telocnemes. It may be supposed, therefore, that in *Arachnactis albidus* and *A. sibogæ* the telocnemes are formed by the fourth couple of mesenteries, and this genus is, therefore, to be associated with *Pachycerianthus*. But the peculiarity is not confined to these two genera, occurring also in the larval genera *Dactylactis* and *Ovactis*, and therefore it has, apparently, more than generic value. It seems to me advisable to divide the acontiferous *Cerianthæ* into two families, according as the telocnemes are the second or the fourth couple of mesenteries, and for the one family I would suggest the name *Cerianthidæ*, while the other may be termed the *Arachnactidæ*, this latter family including the genera *Arachnactis*, *Dactylactis*, *Ovactis* and *Pachycerianthus*.

No stages of *A. brachiolata* and *A. bournei* have yet been studied that are sufficiently advanced to show acontia, and while the mesenteries of the second couple decidedly surpass those of the fourth couple in length in the oldest known larvæ, yet this may be merely a growth condition, the latter couple not yet having had time to acquire their future length relations. However, even if the probability that in these species the telocnemes are the second couple be disregarded, their dissimilarity from

A. albida and *A. sibogæ* in general form and in the order in which their tentacles develop sufficiently demonstrates the necessity for separating them from the genus *Arachnactis*, of which *A. albida* is the type.

The question as to their proper systematic position must be left for future observations to determine, but from what is known of their life histories it seems exceedingly probable that they are larval forms of species of *Cerianthus*. Indeed, Van Beneden (1898) regards *A. bournei* as the larva of *Cerianthus lloydii*, his conclusion being based mainly on the fact that the areas of distribution of the two forms are essentially the same, and, for a similar reason, Kingsley (1904) has suggested that *A. brachiolata* is the larval form of *C. borealis* Verr. There is much probability in these suggestions and the probability will be greatly increased if future observations show that the mesenteries of these two couple possess acontia in these two species of larvæ.

In conclusion, I desire to thank Professor Max Weber of the University of Amsterdam for his courtesy in allowing me to publish in the present paper results that are to a large extent based on material contained in the Siboga collections and which will be more fully considered in a forthcoming report on the Siboga Actinians which is now in course of preparation.

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LIFE AND BEHAVIOR OF THE CUCKOO

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TWENTY-THREE FIGURES

SEVEN PLATES

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1. INTRODUCTION

When the "cuckoo" is mentioned without reservation the gowk or common grey cuckoo, *Cuculus canorus*, of Europe, is commonly referred to, and there is no bird in the world which

has excited more interest among naturalists and people generally, or about which more has been written, and it must be added, too often foolishly written, in both ancient and modern times. This interest is primarily due to its 'clever trick' of foisting its own eggs upon other species of birds, which usually accept them and become foster-parents to their young though at great cost for the price of rearing every cuckoo is the total and invariable destruction of the offspring of the dupe. But this is not the only anomalous conduct in the life of these birds, which seem to break more rules than most of their kind. Of more interest to the biologist and psychologist are the accounts, whether accepted at face-value or not, of the ways in which the cuckoo seems to scheme to accomplish her ends, to smuggle her egg into a proper nest at the proper time, and even to watch the result.

The striking fact that the European cuckoo builds no nest of its own, but steals one instead, that the same bird lays but one egg in a given nest, and that its young are reared by nurses was reported by Aristotle. For over two thousand years since, the question has been repeatedly asked: "Why does not the cuckoo brood?" Speculation has been rife, as is usually the case where the facts are insufficient to trace the history of the origin of a habit or instinct, and a satisfactory answer to the question is not to be found in the many and varied theories and conjectures which have been given from the times of Aristotle and Pliny to those of Darwin and Baldamus.¹ After giving a history of opinion on this subject and a summary of what he regards as the established facts, the latter concludes his long monograph on the cuckoos of the world with the following words: "All answers to the wider questions of *how* and *why*, in my opinion, can be based only on conjectures: and, however clever many of these may be, for exact science they have scarcely any value at all."

For many years the present writer has been looking for an opportunity of studying the instincts and general habits of the American cuckoos, in the hope that in their behavior a key might be

¹Baldamus, A. C. Eduard. *Das Leben der europäischen Kuckucke, nebst Beiträgen zur Lebenskunde der übrigen parasitischen Kuckucke u. Störlinge.* pp. i-viii, 1-226, mit 8 Farbendrucktafeln. Berlin, 1892.

found to open up this apparent mystery, or at least to cast a ray of light upon it, and happily he has not been disappointed.

Darwin,² who has fully discussed the specialized instincts of the *Cuculus canorus*, in the first place accepted the view then often expressed, that the cuckoo did not brood, because she laid her eggs not daily, as in the case of most birds, but at intervals of two or three days, a condition which it was thought would extend the breeding season to undue length.

Since, however, the American cuckoos were found to be in this very predicament, and yet built their own nests and reared their own young, Darwin was evidently constrained to go a step further and conclude that these birds were passing by the same road of natural selection long ago traversed by the ancestors of their European relatives, and that they were now headed for the same goal which the latter had long ago reached. In fine, he believed that our American species were losing their nesting instincts, and that they had already taken the same retrograde steps towards that kind of parasitism which their European relative had so successfully adopted.

So far as we are aware no detailed study of the instincts and general behavior of the American cuckoos in the young and adult state has been previously made, and it is safe to say that had Darwin been in possession of the pertinent facts in their history, he would have reached a different conclusion in regard to their destiny from that which he has presented in his famous chapter on Instinct in the "Origin of Species." Laboring under this influence or that of writers who have misinterpreted certain well known facts in the life history of our birds, Baldamus has even classified the American cuckoos with the non-brooders, in which might be included with equal show of justice such types of exemplary parental conduct as the pigeons or even the domestic fowls. As the following narrative will show, the true answer to the question of brooding or non-brooding is to be mainly sought in the analysis and comparison of the cyclical or reproductive instincts of this and other species of birds.

² Darwin, Charles. Origin of species. Vol. 1. chap. 8, p. 330, New York, 1896.

According to Baldamus, 230 species belonging to the family Cuculidæ have been described in both hemispheres, ranging from the tropics where they are most abundant, to the polar circle, which is sometimes overpassed by the *Curculus canorus* of Europe in its spring migration. Of these, 140 are classed by this writer among the non-brooders, but this number is considerably too great, and but few of them seem to have been studied with sufficient care. Yet, according to Knowlton,³ the 'evicting instinct' (see pp. 178-180) has been recently observed in the young of several species of Australian cuckoos.

North America possesses three genera; *Coccygus* (arboreal cuckoos), *Geococcyx* (terrestrial cuckoos), and *Crotophaga* (including the Ani or Savanna blackbird), and eight species, most of which belong to the Southern States or West Indies. Two only, the black-billed (*Coccygus erythrophthalmus*), and the yellow-billed cuckoo (*C. americanus*)⁴ cover a wide range and are well known in the Eastern and Central United States. All build nests and all brood and tend their young with as much devotion as most birds are able to show.

The present paper deals with the development, instincts, and general behavior of the young and adult black-billed cuckoo but since its relationship with the *Coccygus americanus* is extremely close, it is practically certain that in respect to behavior they agree in all essential respects.

2. METHODS AND OBJECTS OF STUDY

Observations on the life and behavior of nesting birds, and here I chiefly refer to passerine and other land birds of the interior, which are exact, continuous, and fairly complete, can hardly be said to exist, and for this reason, I shall tabulate such results as have been obtained for the black-bill, however incomplete, with considerable detail, only regretting my inability at this time to

³ Knowlton, Frank H. *Birds of the World*. Am. Nature Ser. p. 443. New York, 1909.

⁴ When American cuckoos are mentioned, without further qualification, in this paper, these two species only are referred to.

extend them at either end of the reproductive cycle. To the difficulty of obtaining full and satisfactory records of the free behavior of birds is doubtless due the paucity which exists. To illustrate: before the writer's work, represented in a small part by the present paper, was undertaken, over ten years ago, the cyclical instincts of birds had never been properly studied and analyzed in our commonest species. (Especially as to the terms nos. 3-6 as given in section 5). The field was practically unexplored by both biologist and psychologist. Such common stereotyped behavior as inspection of nest, throat-testing, and shielding, had never been described, but little was known on the subject of *nest-building* as distinguished from description of the completed structure.

With the exception of a few notes on young birds held in captivity, these observations have all been made in the field, at Northfield, New Hampshire, in July and August, 1908 and 1909. My object being to study the correlative instincts of the young and adult in relation to all that could be learned about them in a natural environment, I have followed my usual custom of going out to the birds, instead of taking them into the laboratory. The facts which the laboratory can be made to yield are invaluable, but they belong to a different class from those for which we are now mainly in search, behavior under the usual or normal conditions.

Knowing that the conditions for the study of free behavior in the field, especially with birds where the young and adults are held for a considerable time to a fixed point by the nest, can be made to yield far more to biology and psychology than has yet been realized, it seems to me best to follow such methods whenever practicable, and so long as any new or important result is to be attained.

My general methods of study have been fully described elsewhere,⁵ and I will now only add that aside from making any of the weighings or measurements which may be needed, and testing the reactions of the young under various conditions and at stated

⁵ The home life of wild birds; A new method of the study and photography of birds. Revised ed. New York, 1905.

times, and the use of any chance available means whatsoever, it consists in watching the conduct of young and old at their nests from the concealment of a tent, at a distance of usually rather less than three feet, and making records which shall be as complete as possible. These records, as a rule, cover from six to eight hours daily, and are continued as long as practicable, but can rarely be extended beyond seven or eight days. Three nests of this cuckoo are embraced in the present study, and are referred to by number, when it is necessary to designate them.

By this and similar means I have been able to follow very closely the process of nest-building from start to finish in the Baltimore oriole, the red-eyed vireo, and other birds, and to record minutely the activities of nesting life in over sixty cases, representing over thirty species of native birds, and in many of these as in the black-billed cuckoo, from hatching to approximately the time of flight of the young.

3. THE CUCKOO OF EUROPE

Since it will be necessary to compare the habits of *Cuculus canorus* and of the American cuckoos as closely as possible, I shall endeavour to give here a concise history of the former, emphasizing such statements of fact as seem to be most pertinent and trustworthy, or to call for criticism.

The literature of the European cuckoo is both ponderous and perplexing, and so far as I am aware no fairly complete bibliography, which would doubtless fill a good-sized volume, has ever been attempted. I can only hope that the best, if but a fraction of these writings, most of which can be of no value to the modern student who looks only for exact and reliable data, have come under my eye. Among the older accounts of the last century perhaps the best was that given in 1840 by MacGillivray,⁶ who also figured in his careful manner the digestive and reproductive organs of this bird. Of the more recent histories probably the

⁶ MacGillivray, Wm. A history of British birds. Vol. 3, pp. 105-140, pl. 16, London, 1840.

most reliable and certainly the most elaborate is the work of Baldamus, already referred to, published in 1892, although his earliest communications on the subject date from 1853. Doubtless a year seldom passes without adding something new or old to the life history of this celebrity among birds.

According to Boulder Sharpe,⁷ to write an adequate biography of the cuckoo, one would have to devote a lifetime to the task. This was apparently done by Baldamus,⁸ who reminds the critic that his final work had been revised ten times, and was completed close to his eightieth year. While this observer has few theories to offer, and is plainly intent only on reaching the truth, his references to authorities are meagre, and the conditions under which his observations were made are seldom given. In the following summary I shall frequently translate direct from Baldamus, as indicated, giving my own conclusions in certain cases with such comment as seems desirable.

1. The breeding range of *Cuculus canorus* extends over a large part of Europe and Asia. The birds migrate at night, either singly or in small companies, the males leading by upwards of a week, and tend to return to the place of their birth. In the southward swing which begins rather early (in middle Europe, July-August), the adults precede the young, which migrate independently.

2. The well-known cuckoo note of spring is from the male, the female having a different and much harsher love-call, suggesting the sound of bubbling water. She attracts suitors in numbers, and fights are likely to ensue before pairing is accomplished. (Compare no. 4).

3. The cuckoo formerly built a nest and reared its own young, but probably never does so now. Of the three or more cases reported of brooding cuckoos, two have been thought to rest on mistakes in identification. The evidence for the third, furnished by Adolph Müller, was considered sufficient by Darwin, but is rejected by Baldamus. Müller maintained that the cuckoo

⁷ Wonders of the bird world, p. 301.

⁸ *Op. cit.*, pp. vi, pp. 211-214.

occasionally laid her eggs on the bare ground, brooded them, and fed her young. "This rare event," said Darwin, "is probably a case of reversion to the long-lost aboriginal instinct of nidification."

Nest-building must have been an ancient practice among birds when the cuckoo began to acquire its present habits, and the fact that it takes a lively interest in nests,—that so many of its present instincts and associations have to do with nests, points direct to the conclusion that it once made its own nest, or at least brooded its own young.

4. Although in the pairing season⁹ fights occur among the males, the female European cuckoo is for a time at least polyandrous, there being five or more males, (or suitors) according to conservative estimates, to every female. (Compare no. 2.)

5. "The female cuckoo, with or without a male, and either before or after union, searches for nests of suitable nurses, and when found, watches them from the beginning of nest-building day by day, in order to choose the one most suitable." (Baldamus, and others.) Since we are convinced that the behavior of this bird has been molded by instinct rather than determined by free ideas, we should lay no stress upon choice or motive, as the writers quoted here or in subsequent paragraphs seem to have done.

6. The eggs of this cuckoo are relatively very small, and have thick resistant shells. When taken at random, they are very variable in color, ranging from blue or blue-green, through speckled blue, brown, mottled or marbled brown and gray to nearly plain white.

7. The same cuckoo lays only one egg in the same nest, a statement which has come down from antiquity, and is generally credited, especially by those who accept the following:

8. The same cuckoo always lays eggs of similar color, color-pattern, size, and form, in a single season, and probably during life. According to Baldamus this has been proved to hold true

⁹ Some, like Jenner, have maintained that cuckoos do not pair, and while this view is not supported by more recent observers, the subject is still somewhat vague. There may be variation in this, as in so many other respects.

in one case for three successive years. If two or three cuckoo's eggs are found in the same nest they are thus supposed to belong to different birds, and no case is known where such eggs were similarly colored. Newton and others have assumed that in each individual the color pattern of the egg is inherited, and that the species has become split up into a number of more or less distinct groups or gens, each gens laying eggs of a peculiar coloration.

9. The list of foster-parents or nurses of the young cuckoo has been extended to 119 species. (Sharpe.)

10. The cuckoo, as a rule, lays only in those nests which have eggs similar in size and color to her own, a statement originating with Aelian in the second century, and revived in a modified form by others, especially by Baldamus in the middle of the last. While the proper and intrusive eggs are frequently unlike, it is maintained by Baldamus and other naturalists who have adopted this view, that the cases of similiarity are too numerous to be due to chance. Thus, blue cuckoo eggs, which are very rare have been found in the nests of the pied flycatcher and red-start which also lay blue eggs, but whether found under other conditions or not seems, at present, to be doubtful. Baldamus devotes three colored plates to show the similarity of the eggs of *Cuculus canorus* to those of thirteen different nurses. It must be admitted that the chances of mistaken identity here are considerable, and it seems doubtful if this idea would stand an experimental test, which could be easily made, or that difference in color is of sufficient importance in this relation to be of selective value.

We should like to know how many of the 119 potential nurses of this bird would reject an egg of similar size, whatever its color. We know that many birds will accept anything, especially after beginning to brood, while others will not. Some will try to incubate stones or potatoes, and Blackwall mentions the case of a hawk which tried to sit on a steel trap, placed in the nest over the eggs to catch it by the legs. The uniformly speckled eggs of the cowbird (*Melothrus pecoris*) fare only too well when contrasted with the snow-white eggs of the mourning dove, and the nearly white eggs of vireos, flycatchers, goldfinches, and bluebirds.

The commonest dupes of the cuckoo in Great Britain are the titlark or meadow pipit, the pied wagtail, the reed warbler, hedge sparrow and the robin redbreast (*Erithacus rubecula*), in some of which as in the case of the hedge sparrow the introduced egg is frequently very unlike its fellows. If we assume with Newton¹⁰ that the eggs of certain cuckoo gens have come to resemble those of certain nurse-birds whose nests they habitually steal, through the cumulative effects of inheritance and selection, upon the basis of the advantage of escaping detection thus secured we meet with fresh difficulties, for it is further assumed that birds may be divided into two classes,—those which are readily duped and those which are not, but such a classification will not hold. Expressed in another way this might mean that the parental impulses are stronger at a certain time, as when the eggs are laid, in certain species than in others, and this is true. The question involves, however, too many variables, one of which is fear, and the degree of fear or timidity, being determined in a large measure by experience varies, at different times in the same individual. Many birds, moreover, which would ordinarily belong to the 'wise' or 'timid' class, suddenly become 'stupid' or 'bold' with the rise of the brooding instinct, and the corresponding suppression of fear. This is particularly well illustrated in cedarbirds, rose-breasted grosbeaks, and even in the yellow-billed cuckoos, upon all of which the cowbird has been known to inflict its eggs, though in every case very unlike their nest-mates.

The whole subject of the effect of foreign eggs, placed in other birds' nests, by birds of the same or different species or by man,, has been thoroughly investigated by Leverkühn,¹¹ and Watson¹² has shown how the disposition of the noddy tern is suddenly transformed by the sight and touch of an egg, whether artificial or

¹⁰ Newton, Alfred, and Gadow, Hans. A dictionary of birds, p. 124. London, 1893-1896.

¹¹ Leverkühn, Paul. Fremde Eier im Nest. Ein Beitrag zur Biologie der Vögel. pp. i-xii, 1-212. Berlin, 1891.

¹² Watson, John B. The behavior of the noddy and sooty terns. Pub. Carnegie Institution of Washington, pp. 103, Washington, 1909.

not, which was introduced into its nest. (See p. 224). In a general way it has been found that in certain families and species of birds foreign eggs or other objects are more uniformly accepted than in others. Thus while pigeons will usually accept anything, thrushes are more discriminative, but the behavior of any particular thrush will depend upon its individual characteristics at the moment of introduction, and upon the intensity of the brooding impulses at that time.

Leverkühn, who has tabulated all the experiments, made up to his time, on the behavior of birds towards foreign eggs, placed in their nests (although he has intentionally left the cuckoos out of his account), found that these intrusive objects were received and brooded about as frequently as they were destroyed, or abandoned together with their own proper eggs. His extensive tables embrace 222 species of birds of nearly all the families, observed by many naturalists chiefly in Europe and America, during more than a century, and include 406 cases, where definitive results were noted.

Although Baldamus maintains that all cuckoos' eggs, both native and exotic to Europe show a great similarity to the eggs of their nurses, we do not consider the question to be definitely settled. Meantime we are inclined to regard the variability of the cuckoo's eggs as a lingering expression of a general variability which this bird must have undergone previous to and during the disturbance of its rythmical reproductive activities entailed by the lapse of the impulses to build a nest and tend its young.

11. The cuckoo is said by Baldamus to prefer those nurses, in the nests of which it has itself been reared, a statement obviously difficult, and except in the rarest cases impossible to prove without 'banding' the bird; to lay no egg in suitable nests, if observed by man, or if those nests have been disturbed or touched: to seek to introduce its egg in the absence of the nest-owner, and to carry off the laid egg in bill if, in laying, it has been observed. It would seem that touching the nest could have no possible effect, unless we attribute to the cuckoo the keenness of scent of

a pointer or beagle hound, something unknown to the entire class of birds. Comment on the last remark is unnecessary in view of the following:

12. In open or not too fragile nests, or such as the bird can enter without injuring or destroying them, it lays its egg direct while sitting on the nest-wall. When available nests are inaccessible, or when they are vigorously defended by their owners, it drops its egg on the ground, seizes it in bill and waits a favorable moment for inserting it quickly and unobserved in a suitable nest. (Baldamus.) In want of other appropriate nests, the females will sometimes utilize those placed on or about buildings, not shunning the neighborhood of man. (Baldamus and others.) "If it finds no suitable nest of nurses in its region, it inserts its egg at haphazard in the nests of such kinds as it does not otherwise use, or in those nests in which the egg can come to nothing on account of the degree to which the brooding of the nurse's eggs has advanced, or it lays the egg upon the ground, and troubles itself no further." (Baldamus.)

We regard the action of coming to buildings as simply a variation of the usual practice, as when a robin nests on a porch, or a nighthawk deposits its eggs on the gravel roof of a city house. When this is done, or when the egg is dropped on the ground to be either removed or abandoned, how is it possible to know that the bird has previously made an exhaustive search for the proper nest, and failed in her quest? Some of these actions may be actually due to a lack of proper nests, but are more likely to result from failure to look for the nest, that is to the imperfect attunement or complete blocking of the usual instinct.

13. "The female visits those nests containing its eggs or its young, for the most part in the company but not too close companionship of the male, frequently, each day, and until the young leave the nest. Later they take no further interest in their progeny." (Baldamus and others.) Such statements imply a desire and an ability on the part of the parents to learn the fate of their offspring, and to render it assistance, if needed. No doubt, cuckoos have often been known to remain in the vicinity of a 'stolen' nest, but the question arises whether they have been

correctly identified as owners of the introduced egg, and if so whether such conduct might not be evidence of association or of a recrudescence of parental impulses, as well as of watchful care, directed in the manner implied.

14. To Wetterberg is due the doubtful observation that the cuckoo turns the eggs of the nurse with her bill, as the opportunity offers, and pushes her own egg into the center of the nest. Such actions, if performed, would surely be useless, since birds turn and stir their own eggs with bill or feet, when entering the nest or when incubating. I have repeatedly seen the black-billed cuckoo grasp an egg with her foot when settling down, and draw it under her, and have photographed the great herring gull in the act of turning her eggs with the bill, and both that bird and the cuckoo in the act of moving an egg with the foot, and placing it under the body. If the observation referred to above has any value, it shows the persistence of what is undoubtedly a very ancient and useful instinct.

15. The cuckoo's eggs are laid according to some observers at intervals of two or three days, and of six or seven days, according to Baldamus, and to the number of five or six, and rarely seven in the season, the ovaries and oviducts closely resembling those in other birds. Eggs are found in middle Europe from the end of April to the beginning of July, but for the most part only during the second half of June, but rarely to the end of July. (Baldamus.)

16. According to most observers the cuckoo's egg is thought to hatch about twenty-four hours earlier than those of most nurses. A similar precocity was attributed to the eggs of our cowbird by Alexander Wilson, who was the first to describe its peculiar habits. No exact determinations, under proper control, have yet been made upon this interesting question.

17. *Fate of the eggs and young of the foster-parents.* All authorities agree that as in the case of the nest-mates of the cowbird, the entire progeny of the cuckoo-nurse is destroyed, but there is a curious disagreement between observers in Great Britain and on the Continent as to how this is brought about.

English naturalists, from the classical observations of Dr.

Jenner,¹³ published in 1788, have attributed a series of highly specialized instincts to the young cuckoo, in accordance with which it burrows under any eggs or nest-mates which may be present, gets them on its broad and depressed back, climbs or pushes its way up the steep side of the nest, and shovels them all out over its rim. Eggs and young are eventually treated in this manner as often as they are returned, and if, as rarely happens, two cuckoos are hatched in the same nest, they struggle together until the weaker of the two is pitched out in the same manner. According to the accounts to be referred to below, this response appears at any time between the first and third day, when the little cuckoo is blind and naked, and dies down when the bird is from ten days to two weeks old, after which anything is tolerated in the nest.

It has been sometimes stated that the first confirmatory observations of Jenner's controverted statements were first made by Mrs. Blackburn, and published in 1872,¹⁴ and her striking drawing of¹⁵ the infant cuckoo in the act of expelling a young meadow pipit from its nest has been widely copied. It should be noted, however, that the Jennerian story did not have to wait so long for supporting evidence, for it was fully sustained in 1802 by Col. Montagu¹⁶ in his excellent Ornithological Dictionary, in which we are moreover told that his own observations were actually made before those of Jenner, that is before June 18, 1787. Confirmatory observations were again made by John Blackwall, before 1834, and fully described in his excellent, but little known "Researches in Zoölogy."¹⁷ Again the original account was supported in the fullest manner in 1838¹⁸ by the correspondent of Wm. MacGillivray,

¹³Jenner, Edward. Observations on the natural history of the cuckoo; in a letter to John Hunter, Esq. Philosophical Transactions, vol. 78, pt. 2, pp. 219-237. London, 1788.

¹⁴"J. B." Cuckoo and pipit. Nature, vol. 5, p. 383. London, 1872.

¹⁵First appearing in "The pipits", Glasgow, 1872, and later in "Birds from Moidart," Edinburgh, 1895.

¹⁶Montagu, Colonel G. Ornithological dictionary of British birds, 2d. ed. by James Rennie., p. 118. London, 1831.

¹⁷Blackwall, John. Researches in zoölogy, London, 1834; 2d. ed. London, 1873.

¹⁸*Op. cit.*, vol. 3, pp. 128-131.

Durham Weir, who then gave one of the best accounts of the behavior of the nestling cuckoo which we still possess.

Those who rejected Jenner's account relied either upon negative evidence or upon analogical reasoning, as in the case of Charles Watterton, whose bitter attacks upon Audubon, mainly rested upon the same fallacies. "The young cuckoo," said Watterton "cannot by any means support its own weight during the first day of its existence. Of course, then, it is utterly incapable of clambering rump foremost up the steep side of a hedge sparrow's nest, with the additional weight of a young hedge sparrow on its back. The account carries its own condemnation, no matter by whom related or by whom received."

Blackwall, who placed a young cuckoo, hatched by a titlark, in the nests of other birds and watched the eviction of their eggs and young, made this significant remark: "I observed that this bird, though so young, threw itself backwards with considerable force when anything touched it unexpectedly." It has been stated that while this cuckoo will inevitably evict a live bird it permits a dead one to remain, but this seems likely to be an error. The peculiar hitching movement by means of which this blind nestling is able to get rid of its nest-mates possibly arose as a reflex response to a contact stimulus of a disagreeable kind. The repeated stimuli call into play both legs and wings, and indeed the whole body, and after one trial at evicting an egg, Hancock saw the little cuckoo fall back into the nest as if in a state of exhaustion; two hours sometimes elapsed before any fresh attempt was made. In every case where this highly specialized instinct has been shown to be well developed, we are inclined to believe that any object simulating an egg or young bird would seldom fail to awaken this response, when the evicting instinct is at its height, although the stimulus afforded by a struggling live bird would be greater than that of a dead or passive object, whether nestling or egg. This would seem to explain the observation by Jenner that after the third day, when this instinct is on the wane, an egg is tolerated when an active bird would be expelled.

We come now to consider a series of contrary statements made

by Baldamus and others, as to the way in which fate overtakes the eggs and young of the nurse. "The female cuckoo removes and hides the eggs of the nurse, after the young parasite is hatched, and has been adopted, and in doing this it is attended by the male who keeps in the vicinity of the nest." (Baldamus and others.) "In nests which it cannot readily reach, the young of the nurse sometimes grow up, but are often suffocated or starved out by the young cuckoo, and are later removed by their own parents for the sake of cleanliness." (Baldamus.)

It would thus appear that the evicting instinct of young cuckoos is neither perfect nor universal, and that the young of the foster-parents are often treated precisely as in the case of our cowbird. If true that the adult cuckoo ever removes the unbroken eggs of the nurse, when its young fails to do so, it is evident that this is not regularly done, but it would be useless to speculate on the significance of such an extraordinary act, without more precise knowledge of the conditions under which it is supposed to occur.

18. The European cuckoo is now generally absolved from the stigma of eating the eggs or the young of other birds. The eggs which have been found in the bill or oesophagus of a shot bird, are supposed to be either its own which it was carrying to some nest, or those of a nurse (see section 12) which it was in the act of removing. (Baldamus and others.)

19. Adult cuckoos, when the opportunity offers, feed freely on hairy caterpillars, so universally rejected by other birds. They have large, thin-walled stomachs, the mucous membranes of which are often 'furred' with the sharp, stiff hairs or setae of these insects. They also take a great variety of other insect prey, in the adult and larval state, a few berries, and incidentally a little sand and very small pebbles.

20. The European cuckoo, coming from a smaller egg, is less advanced at birth than in the case of the American species. Incubation of the cuckoo's egg has been known to last two weeks (Weir: by titlark, May 23-June 6); the young are born not only blind, but according to most accounts, without any trace of

feathers.¹⁹ Like our birds they reach the 'quill stage' in about a week. The young remain in the nest for upwards of three weeks, instead of climbing out on the seventh day, as in the case of the American black-bill. We should not expect to find a climbing stage present, and none has been described. In the case recorded by Weir, nest-life was prolonged to twenty-four days, and it probably lasts until the cuckoo is able to take short flights. After the nest is abandoned the cuckoo is assiduously attended for a considerable time by its nurses, as with the cowbird, and has been known to eat freely of hairy caterpillars shortly after becoming independent.

21. The sense of fear seems to be expressed in much the same way in the European cuckoo, as in the American species, with the exception that in the former it is longer deferred and does not lead to a premature desertion of the nest. More observations on this subject are needed, but the following remarks of Jenner²⁰ will be read with interest: "Long before it leaves the nest," the cuckoo "frequently, when irritated, assumes the manner of a bird of prey, looks ferocious, throws itself back, and pecks at anything presented to it with great vehemence, often at the same time making a chuckling more like a young hawk. Sometimes, when disturbed in a small degree, it makes a kind of hissing noise accompanied with a heaving motion of the whole body." According to Jenner's account the cuckoo expresses its fear in a manner calculated to inspire fear in its common enemies, and with the important exception noted, much in the manner of its American relatives.

¹⁹ Mrs. Blackburn's drawing (*Op. cit.*, p. 110), which is far from correct in details (as in the head and foot), shows very little trace of down feathers.

²⁰ *Op. cit.*, p. 234.

4. HABITS OF CUCULUS CANORUS AND THE BLACK-BILLED CUCKOO COMPARED

For a better comparison of the European and American cuckoos, some of the significant facts in their life-histories are here presented in parallel columns, further details on the habits and behavior of the American species being given in the following sections.

CUCULUS CANORUS

COCcyZUS BYTHIROPETHALMUS

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| 1. <i>Migration</i> : Spring order: males precede females by about a week; fall order: adults, followed by the young. | The same. |
| 2. <i>Nocturnal habits</i> : Migrate at night and frequently active and calling all night. | The same |
| 3. <i>Length of adult</i> : 12 in. | 11.83 in. |
| 4. <i>Weight of female</i> : 4½ oz. | |
| 5. <i>Size of egg</i> : Extremes, 24-20 × 17.4-15.7 mm; | 29.27-22.35 × 22.86 - 18.54 mm; |
| average, 22 × 16.5 mm. | 27.23 × 20.53 mm. |
| 0.87 × 0.68 in. | 1.07 × 0.81 in. |
| 6. <i>Weight of egg</i> : 4-5 g. | 6.84 g. |
| 7. <i>Ratio of body-weight to egg-weight</i> : 33:1. | |
| 8. <i>Egg-shell</i> : Hard and thick. | Thin and fragile. |
| 9. <i>Color of egg</i> : Variable. | Light blue; but rarely mottled or marbled. |
| 10. <i>Number of eggs</i> : 6 or more (doubtful). | 2-7 to the litter, and possibly two litters. |
| 11. <i>Succession of eggs</i> : At intervals of 6-7 days (Baldamus). | Irregular; daily, or more commonly every other day. |
| 12. <i>Incubation</i> : 2 weeks, or less; by nurses. | 2 weeks (?); by both parents. |
| 13. <i>Nest</i> : Stolen; egg either laid in nest of a nurse, or on the ground and carried to a nest in bill. The same cuckoo lays but a single egg in the same nest, and it often resembles the eggs of the foster-parents in size and color. | Built in the form of a saucer-shaped platform of twigs and leaves; well protected and concealed. Eggs sometimes laid in another bird's nest, or removed in bill to another nest of their own, when the first has been disturbed. |

CUCULUS CANORUS

COCCYZUS ERYTHROPERTHAI MUS

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| <p>14. <i>Young at Birth:</i> Blind, without trace of feathers (v. 20, Sect. 3); legs large and strong; restless; sometimes responds to contact-stimulus, by throwing itself backwards, and in this way ejects nest-mates; response awakened from 1st to 3d day, and lasts about a week.</p> <p>15. <i>Quill Stage:</i> reached at one week old; instinct of fear probably longer deferred.</p> <p>16. <i>Preening or Combing Instinct:</i> probably present, but without the effect shown in the American species.</p> <p>17. <i>Age at Leaving Nest:</i> 21-24 days, when ready for flight.</p> <p>18. <i>Climbing Stage:</i> None.</p> <p>19. <i>Age at flight:</i> About 21-24 days.</p> | <p>Blind, and dry with black skin sprinkled with white rudimentary down (feather-tubes); legs very large and strong; able to raise itself when holding to twig, and support its weight with single toe; grasping reflex very marked.</p> <p>The same, when it shows fear.</p> <p>Appears at about beginning of 7th day: feather-tubes "combed" off entire, over large part of body, and in a few hours.</p> <p>7-8 days, or about 2 weeks before flight.</p> <p>Begins upon leaving nest and lasts about a fortnight.</p> <p>The same, or longer.</p> |
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The facts tabulated in the preceding paragraphs show very clearly that while the European and American cuckoos agree in the broad outlines of their behavior, and even closely at certain points, they diverge widely at others, and chiefly in the following respects: (a) The cuckoos of Europe have lost the nest-building and brooding practices, and such parental instincts as they have retained have become directed mainly to the care of the eggs under peculiar conditions; (b) new instincts have arisen through modification of the old, or the passage of the old into new channels; (c) The eggs have become reduced in size, highly variable in color, and have acquired harder shells; (d) the young have correlatively lost certain former instincts, have become modified in respect to others, or have acquired new ones, the most striking of which is the "evicting instinct," as it may be called by means of which they get rid of their nest-mates. There has probably been a retardation of the rise of fear in the young, in consequence of which it has been enabled to remain longer in the nest of a nurse, but experimental evidence here is defective.

The parental or cyclical impulses of the American species are irregular at one important point only—the production of eggs—and any disastrous results likely to arise from this cause have been annulled by the development of new instincts in their young, exhibited in the climbing stage, and the preparation for it to be seen in the strong grasping reflex, precocious muscular development, the “combing” instinct, and comparatively sudden appearance of the feathers on the seventh or eighth day.

5. CYCLICAL INSTINCTS

The behavior of birds is determined and expressed by a large number of recurrent instincts, some of which are of commanding importance and most of which are of ancient origin. For the sake of simplicity, their instincts may be divided into two classes; namely: (1) The Continuous Instincts, which are necessary for the preservation of the individual, such as preying, fear, concealment, and flight; and (2) Cyclical Instincts, which are requisite for the maintenance of the race. By cyclical instincts we mean those discontinuous, recurrent tendencies to action which attend the reproductive cycle, and with this understanding it will be convenient to speak of parental instincts, without specification in each case.²¹

The cyclical or parental instincts as a rule recur with almost clock-like precision, in spring or summer, in the northern hemisphere, and with repetitions within the breeding season in certain species. Fear, which is primarily due to inheritance, is subject to continual modification through experience, and may even lapse completely; it not only modifies the cyclical instincts, especially at the time of their emergence, but may be completely blocked and annulled by them at a later period.

²¹ For abstracts of earlier papers on this subject, see: Analysis of cyclical instincts of birds. *Science*, vol. 25, p. 725, 1907, and The blending and overlap of instincts, *Science*, vol., 25, p. 781, 1907; also Instinct and intelligence in birds. *Pop. Science Monthly*, vol. 76, pp. 532-536, and vol. 77, pp. 82-97, 122-141. New York, 1910. Growth curves and other records of cuckoos are given here.

The parental instincts are further modified at every point by intelligence, and if it be inferred that since we are here dealing largely with the instinctive activities of birds, we assume that their behavior is due to instinct alone, the impression would be grossly misleading and thoroughly false.

The reproductive cycle is made up of a series of acts or chains of actions, which follow in a definite succession. Eight or more terms may be recognized, but the classification is unimportant, so long as it is observed that they are *serial* and *harmonious*, and that anything which profoundly disturbs their normal attunement is disadvantageous, and may lead to disaster. If the disturbance is of a fundamental and permanent character, new adjustments in the series must follow, if the species survive.

The cycle may be graphically represented by a number of nearly tangent circles, each of which stands for a distinct sphere of influence or for a subordinate series of related impulses, as given in the simplified formula:

1. Migration;
2. Mating;
3. Nest-building;
4. Egg-laying in nest;
5. Incubation and care of eggs;
6. Care of young in nest;
7. Care and "education" of young out of nest;
8. Migration.

A partial descriptive analysis of the successive terms of the cycle and of the correlated instincts of parent and child are given in the table which follows, but it must be understood that in a complete catalogue of the separate or related instincts of wild birds, this list would be extended to much greater length, and that the selection of terms, especially Nos. 6 and 7, is conventional.

TABLE 1

Analysis of reproductive cycle

PARENT

YOUNG

1. *Migration* to breeding range, or place of birth.
2. *Courtship, and mating*; often attended by song and peculiar antics, especially in the male.
3. Nest-building.

{	<ol style="list-style-type: none"> (a) <i>Selection of nest-site</i>, or resort to old, by one or both, and often attended by guarding and fighting instincts. (b) <i>Building of new nest</i>, or adaptation of old, by one or both birds, and often with instincts of guarding, pugnacity, and concealment.
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4. *Egg-laying*; usually at daily intervals, in completed nest, and as before often with guarding, fighting and concealment.
5. *Incubation* or brooding instinct; beginning at a certain time, and with its gradual rise often completely allaying fear; as before attended by instincts of concealment guarding and pugnacity. The attendant care of eggs embraces a variety of instinctive acts, sometimes recurrent, as removal of eggs in bill, inspection of eggs, stirring of eggs with bill or feet, cleaning nest by removal of broken eggs or shells, shielding eggs from heat, and sometimes hiding them with covering of leaves.

6. *Care of young*, embracing regular recurrent instinctive acts, as follows: (a) feeding young; including capture and treatment of prey, return to nest, (pause), (call-stimulus), testing reflex response of throat of young, watching for response or swallowing reflex, (pause); (b) inspection of young and nest; (c) cleaning young and nest; excreta eaten before or after removal, or disposed of in a special manner, besides incidental or irregularly recurrent acts, such as brooding, shielding or spreading over young, whether sitting or erect, bristling, puffing, preening, gaping, yawning, stretching, guarding and fighting. Initial responses of young at moment of hatching, or shortly after, including grasping reflex of feet, elevation of head and opening mouth at first evoked by a variety of stimuli, but gradually limited by association with the nest or with the sight or sound of the parent; swallowing reflex in response to contact of bill of parent with the food inserted by bill in the mouth or more commonly in deep part of throat, in altricial birds; call-notes, and later alarm notes; gaping, yawning, stretching, and spreading in response to heat, burrowing under feathers of old bird or under nest-mate; preening and 'combing' feather-tubes, fear and bristling, pecking, flapping and flight. Special instincts acquired by young of cuckoos and cowbirds, and certain precocious species which are not fed long at the nest.
7. *Care and 'education' of young*. Guarding, fighting, feeding, and luring or enticing the young often leading incidentally or indirectly to a process of education, as in gulls, where the food is regurgitated on the ground, and the young getting it in this way, learn to look to the ground as its source. Flight, fear; seeking prey; giving call, and alarm notes; following, crouching, and hiding; performance of various acts through imitation.
8. *Fall migration*. Singly, or in company with individuals of the same or of different species, to winter station. With adults, or independently.

The descriptive formula or catalogue given above is a composite, but with slight changes will represent the related instincts of parent and child as they normally appear in the breeding cycle of the typical altricial and of many precocious birds. Normally

the bird passes from center of influence 1 to center 2, 3, and so on, to the end of the cycle. It remains under the influence of a given instinct or series of instincts, such as nest-building, incubation, or care of young, until its impulses in each direction have been satisfied, before entering a new sphere, or being swayed by new instincts. When the correlation or attunement is relatively perfect the instincts of parent and child fit like lock and key. To change the figure, like clocks beating synchronously the instincts of parent and child are generally in harmony, but one of the clocks occasionally gains or loses, stops or runs down: one term is liable to be weak or to drop out altogether, so that there is a gap in the series, which leads to eccentric behavior and often proves serious to eggs and young. On the other hand, one term may be unduly strengthened, like nest-building or incubation, and a preceding or following term correspondingly weakened. Such disturbances which are more common than is generally known in all birds, vary with the individual, but are more prevalent in certain families and species than in others. They have been described in a former paper under the head of the "blending" and "overlap" of instincts,²² wherein it was shown that they account for many puzzling and hitherto unexplained eccentricities of conduct such as repairing the old nest or building a new one at the close of the breeding season (in eagles, fish hawks and gulls), temporary suspension of nest-building and dropping the eggs on the ground (in ostriches, cowbirds, and many other species) leaving the young to perish in the nest and starting on migration (most frequently noted in swallows), building supernumerary (in robins, wrens and other species) and superimposed nests (in warblers with cowbirds' eggs), and other anomalous actions, which have been subject to general and varied misinterpretation.

In many species, the cycle is normally repeated one or more times within the season, and in most cases when the series is broken at 3, or at 4, by fear, or by loss of the eggs, the cycle is begun anew at 3. In the most aberrant cases of behavior as in some of the *Megapodes* of Australia and the East Indies,

²² *Op. cit.*, p. 781.

which either bury their eggs like a turtle in the sand, or construct a kind of "mound-nest" for an incubator, and certain cuckoos and cowbirds of both hemispheres, which prey as it were, upon the instincts of other birds, the parental impulses have become profoundly modified, and diverted into other channels and in a peculiar manner. On the other hand the American cuckoos follow the usual schedule as completely and with nearly the same regularity as the American robin, or any other passerine bird.

6. THE NESTS AND EGGS OF AMERICAN CUCKOOS

Following in a general way the terms of the reproductive cycle, outlined in the preceding section, we shall now give an account of the behavior of the black-billed cuckoo, particularly of the adult in relation to the young, with the addition of such notes on what may be called their general habits, as have been observed and are worthy of record.

Migration takes place in April and May, and in the fall in September and October. The breeding range is very wide, extending from at least 35° to 71° north latitude in places, and from the eastern slopes of the Rocky Mountains to the Atlantic seaboard. These cuckoos migrate at night, possibly the males in advance of the females, but this does not seem to have been positively determined. The northern tier of States is reached in early May, with the main stream of summer resident birds. The call and alarm notes of both sexes are similar, though according to some acute observers different in the two species, and seem to vary considerably in accordance with the distance from the ear, and the emotion of the bird which utters them. The loud and often prolonged *kow-kow-kow-kow* note, is the common responsive call frequently heard both at and away from the nest, and has given origin to the common names of *kow-kow*, or of *rain crow*, in supposed relation to the weather. It seems to be uttered only when the birds are perched, and when heard in early spring, is probably the love-call of the male. The birds, though possessing a hawk-like profile and appearance, for which their kind has been

celebrated from antiquity, are on the whole shy and retiring, frequenting copses, thickets, and bush-grown pastures, near to water, where their insect prey abounds. With rapid flight they, glide stealthily and noiselessly about, "often resting motionless as statues for a long time." They are active all night, and while in camp at Lovell, Me., August 7, 1909, I heard the call, *koo-kúk! koo-kúk! koo-koo-kúk!* from this bird at intervals from 9 p.m. until 12.30 a.m., long after the whippoorwills had rested. Gerald Thayer has²³ observed at Mt. Monadnock, N. H., not only the usual nocturnal activity as shown by their responsive *kow-kow* note, but what seemed to be a regular practice, even on the darkest nights, of flying high, possibly as high as four hundred or five hundred feet, and uttering a series of rolling guttural notes, resembling their usual alarm, even when rising above the ridges of the mountain at an elevation of 3,000 feet.

Though frequently denied, it is undoubtedly true that these cuckoos destroy many eggs and young of other birds, and I will mention a case in point. Twice (July 2, and 3, 1907) at my home in the country, near Cleveland, Ohio, I have seen a black-billed cuckoo, chased by robins from an elm tree, in which these birds were known to have a nest, and bearing in its bill each time what looked like a small and very immature nestling. These robins, sounding their shrillest alarms, and darting at the retreating cuckoo, followed it for nearly a quarter of a mile. Later the same season (July 18), a bird answering to the description of this cuckoo was seen near the same spot flying off with an egg in its bill, this egg being undoubtedly pierced.

The black-bill nests from late May to late August, laying from 3 to 7 eggs to the litter, and probably sometimes produces two broods in a season; it certainly does so, when upon the disturbance or destruction of its eggs, it abandons a nest. A nest has been taken as early as May 7th, in Mount Carmel, Ill., and as late as September 10th, at Lockport, N. Y., where the frosts had been severe enough to destroy vegetables on the two pre-

²³ Thayer, Gerald H. Mystery of the black-billed cuckoo. Bird-lore, vol. 5, pp. 143-145. 1903.

vicious nights. The first of these nests contained eggs, and the last two recently hatched young.²⁴

The nests of the black-bill are commonly placed at a height of 4 to 5 feet from the ground, but have been found as high as 18 feet, and as low as 25 inches (nest no. 2, fig. 9). They are essentially shallow, saucer-shaped platforms, or concave floorings of coarse twigs, leaves and catkins, of rather greater bulk and better construction than in the case of the yellow-bill, and are usually well concealed and protected from the wind. At Northfield, N. H., we have found them only in saplings of the white pine, and in thorn and wild apple bushes. The act of nest-construction has not been witnessed, but presumably both sexes take part, as is often the case when, as in the present instance, both share in incubation and in the care of the young. One of these nests which was particularly examined had an inner diameter of 4 inches and an inner depth of $\frac{3}{4}$ inch, the greatest diameter being 7 inches, and total height $2\frac{1}{2}$ inches. It consisted of a scant foundation of old brake-leaves, interstratified with coarse twigs, and surmounted with finer twigs and a topping of pine needles, dead leaves of the birch, brake, and willow catkins. Another nest of the season, examined on June 28th, contained the shell of the last egg hatched, and had essentially the same structure, with the addition of pulled grass, and a larger mass of willow catkins, which formed about one-half of the nest materials. I estimated the whole to represent about 150 loads, brought in the bill of one or both birds. Further, judging from the form and symmetry of these nests, the cuckoo practises instinctively both the molding and turning movements, so marked in all species with typical cup-shaped inner walls, though in this case with very meager results.

The eggs (v. table 2 and fig. 1) are nearly oval; they have thin fine-grained shells, and according to Bendire, vary in color from Nile blue to pale beryl green; very rarely an egg is marbled, "caused by different shades running into each other," an illus-

²⁴ Bendire, Charles. Life histories of North American birds. Smithsonian contrib., vol. 32, p. 29. Washington, 1895.

tration of which is figured in the work quoted above (pl. 5, fig. 3). This egg, which was taken in Montana, by Bendire, June 25, 1885, is particularly interesting since it not only illustrates a sporadic variation in color, so marked in the European cuckoo, but also because its owner, when first disturbed on June 22, built a new nest and transferred this egg to it in a space of time not exceeding two days. The occasional transference of the egg from nest to nest, after the first has been discovered, had been noted by other observers, but we should desire fuller evidence before accepting the fact that the young are also moved about in a similar manner. At all events this undoubted transfer of egg in bill, however rare, is a very important fact in enabling us to evaluate the peculiar instincts of the European cuckoo.

7. BEHAVIOR OF BROODING CUCKOOS

The nest (no. 3) here referred to was placed on one of the whorls of a small pine sapling, about three feet from the ground, and when found on July 19, 1909, contained three incubated eggs, and one young bird at least twelve hours old. The conduct about to be described, with greater or less variation, is characteristic of many brooding birds, and illustrates the depression of fear which follows the rise of the brooding instinct.

When the sitter, which we assumed to be the female, was approached to within a distance of five or six feet, she slid off quietly, and with tail spread, flying low, lighted in a small tree close by, where she remained for some minutes, ducking head and flipping tail constantly; she then disappeared, and gave the familiar alarm, at times sounding like *koor-uck-uck-úk*, or like *kur-ut-ut-út*, which no attempt at syllabication can perfectly represent. At this nest the sitting bird had already acquired the habit of facing in a definite direction, and this position was steadily maintained during the nine successive days that observation lasted.

On the day following we again tried to ascertain how near we could approach the sitting bird without disturbing her, by moving

slowly and quietly towards the nest. As you get 'dangerously' near, her head begins to rise, and from a nearly horizontal position, as marked by the bill, it is tilted through an angle of about 45° , the same kind of response as that seen in the cedarbird, the bittern and many other species. In the cedarbird the position assumed is so bolt upright and so rigid as to leave no doubt that it serves as a means of concealment. In this way I approached as near to this cuckoo as it was possible to go or until her eye was twenty inches from my own. The iris of the black lustrous eye was distinctly reddish brown, and across it the nictitating membrane could be seen momentarily flitting, like a focal plane shutter, at an estimated speed of about $\frac{1}{15}$ of a second or much less rapidly than in the case of a bird like the domestic pigeon. The respirations, as registered by the moving bill, tail, wings, and breast-feathers, were rather difficult to follow, but approximated 100 per minute. While watching the same bird under other circumstances, the records varied from 80 to 106. In a brooding female redwing blackbird on a hot day I have seen the respiration rise to 160 per minute.

With every movement of my head the head of this bird would move, as if to keep the object in clearer view. The heaving movements of another character, indicated as was already evident from the behavior noted, that a second young cuckoo had been hatched, and this proved to be the case, when after twenty minutes of close inspection, this bird finally withdrew, and gave the usual and emphatic *koor-uck-uck-úk* alarm.

The brooding instinct in this cuckoo has a gradual rise during a period of about three days, and then rapidly, like a fever which has run its course, recedes. In this instance the climax was reached on July 22, when the nest contained three young and one addled egg.

From birth to the adult state four distinct stages are to be distinguished in the life of this cuckoo:

1. *Period of Infancy*, from birth to the age of 5 days.
2. *Complete Quill Stage*, attained on the 6th to 7th day.
3. *The Climbing Stage*, reached on the 7th to 9th day, when the

nest is summarily deserted by each half-fledged young bird in succession.

4. *The Flight Stage*, when the young, fully fledged, leaves the neighborhood.

8. HATCHING OF THE CUCKOO AND INSTINCTS OF YOUNG AT BIRTH

When the female cuckoo slipped off her nest, on July 20th, as detailed in the preceding section at exactly 12.10 p.m., she left two young, two eggs, one of which was pipped, and the shells of the last bird hatched. While I was making the necessary notes, the pipped egg cracked open along its lesser circumference, and left the young (bird no. 3) lying on the larger half-shell, which was thus found a short time later.

As is well known, birds commonly leave the egg wet with the amniotic fluid, but in this case while the egg-membranes were slightly damp, the little bird was perfectly dry, and soon began to struggle out of its shell. To the shell-membrane and pale thin allantois was attached a residual milky white mass representing without doubt the remains of the albumen and excreted substances.

This cuckoo at birth was $2\frac{1}{2}$ inches long, and weighed $7\frac{1}{2}$ grams (fig. 1). It differs from the young of other altricial birds in the following particulars; the skin is dull, coal black, sparsely sprinkled with snow white "hairs," the feather-tubes of a down, which never unfolds. These conspicuous hair-like tubes are distributed over the various feather-tracts of the body; being most numerous on the back, head, wings, and thighs; they vary in length from 3 to 10 millimeters, and are longest on the back and thighs, (fig. 21 *a, b*).

These primitive feather-tubes, are later pushed out by the tubes of the contour-feathers in the usual manner, thus giving to the latter a peculiar flagellated appearance before they unfold (fig. 21, *d*). The legs and bill are blue-black in color, and exceptionally strong, and this infant is vigorous and enduring to a remarkable degree, being able to withstand excessive heat, hunger, and general neglect beyond the young of most wild birds. It can hang

suspended by a leg or a single toe, for upwards of fifteen seconds, and with both legs can raise itself to the support, or until its bill is over the twig, a feat which probably no other altricious bird in this part of the world can perform at birth (figs. 2 and 3). In a very short time it is able to draw itself on to its support, and even to raise itself with one leg, which implies an extraordinary muscular development.

The real significance of this athletic ability is clearly seen when we come to consider the equally precocious climbing instinct, for which it is a direct and necessary preparation. Though born blind, and essentially naked, the young cuckoo is probably in proportion to its size, the strongest, hardiest, and most enterprising altricious bird in North America.

A. Initial reactions at birth

The most striking reactions and powers displayed by the young at birth or shortly after are as follows: (1) The grasping reflex; (2) food-reactions; (3) initial call-notes, and (4) power of orientation.

The grasping reflex (1), which is highly characteristic of the passerine and various other orders of birds, is developed in a marked degree in the young cuckoo. The bird commonly lies flat in its nest at this early period with toes clenched, holding to the twigs or nest-lining with firm grip. If forcibly removed, it is liable to pull its nest to pieces, rather than let go, and will sometimes seize and drag out one of its fellows, reminding us of lobsters and crayfishes, in handling which we are likely also to get a living chain.

When the claws of this young bird finally relax, they open and close very rapidly, and this reaction continues until checked either by fatigue or the satisfaction of finding any object to grasp. The contact stimulus afforded by a solid body alone satisfies this powerful reflex, which from the moment of birth is of the greatest service to the young bird, not alone in holding it to its twig-nest, but in developing the muscles of the hind limbs and body generally and thus preparing it for the climbing stage soon to follow. It

must be considered in relation to all the other special instincts and adjustments, in consequence of which these cuckoos have become as completely adapted to the conditions of their life as have the young of any New or Old World species

The human infant is credited with the ability of sustaining its own weight with its arms, but as we have seen, the infant cuckoo can not only sustain its weight with one or both limbs or by a single toe for an extraordinary length of time, but shortly can perform the remarkable feat of drawing itself up with the muscular power of one leg.

B. The food-reaction and its modification through association

The food-response, which has the nature of a chain-reflex, is a complicated act, in the course of which the head is elevated, and the mouth opened wide, revealing in this species a dull red mouth cavity, marked in a highly peculiar manner. A series of symmetrically arranged snow white spots or pads, converge about the glottis and internal nares, some 14 in number, nearly all with the exception of a prominent spot on the tongue being confined to the palate, (figs. a and 5) the whole presenting a very peculiar appearance, and suggesting the ornamented throat of the nestling of the Gouldian weaver finch (*Poephila gouldiae*) from Australia, figured by Sharpe²⁵ but without colored wattles on the gape.

This complex performance, which represents the simplest sign-language of the hungry bird, appears as a uniform chain-reflex, and is as predictable, and seems to be as mechanical as the response of an electric bell. It does not, however, long remain in this unmodified state. Though at first called into play, so far as can be ascertained by an external stimulus alone, as by jarring, sound, and possibly by the action of the air, and as often as any stimulus acts, within the limits of fatigue, from the moment of birth this reaction becomes modified intermittently (*a*) by hunger, and steadily (*b*) by association with the parent and nor-

²⁵ *Op. cit.*, p. 116.

mal environment. Indeed it becomes so completely linked to parent and nest, that after the seventh day, it can seldom be artificially evoked, in spite of hunger by any of the usual mechanical stimuli whatsoever. So marked is this character that in early life at the nest when the variable a is known, the age of the young bird can be approximately gauged by the nature of the food-response alone.

We should add here that shortly after birth muting begins to follow the taking of food quite regularly, and becomes as stereotyped as any other phase of behavior. The nestling strives to

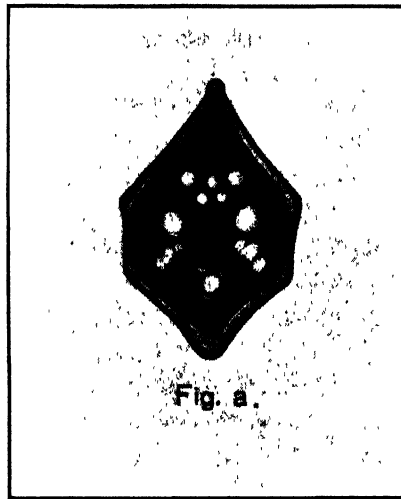


FIG. a "Target" of cuckoo at birth, showing symmetrical pattern of white spots or pads on palate and tongue, when mandibles are opened wide in food reaction. See fig. 5

raise the hinder end of its body, which is turned outward, so that the sac, when not taken direct by the parent the moment it leaves the cloaca, will fall outside of the nest or upon its margin.

During the first 24 hours the variable to be mainly considered in relation to the food-response is hunger, and when this element is known, the former can be predicted with precision, whatever the environment, within the limitations of bodily fatigue. From

the second to the fifth day the response becomes more and more uncertain, when the bird is removed from its nest. Then at the sixth or seventh day, association has become so perfect that this bird will starve while away from the nest, rather than open its mouth.

I have taken a 7-day-old cuckoo from its nest, and kept it 25 hours without being able to get a single food-reaction, whatever the nature of its treatment. In order to feed it at all it was necessary to pry open its mandibles, and force the food into the gullet, and then most of it would be regurgitated. But the very moment this same bird was returned to the nest, and its feet touched the familiar twigs, it expanded as if by magic into a new creature. Immediately erecting its feathers, swelling to almost double its former size, standing erect, with head and neck up-stretched, mouth open wide, wings spread and vibrating, the whole body trembling as with deep emotion, and giving its loudest guttural call, this bird not only presented a picture in striking contrast to what had gone before, but offered a unique example of the force of habit and the power of association. In this respect, however, the cuckoo is by no means peculiar.

The initial call note (3) is given practically as soon as the bird is hatched, and in precocious species like the gull and domestic fowl while still in the shell, and either before or after this is pipped. In the cuckoo it is at first a feeble grating note or grunt, like *kek*, which gradually increases in vigor until it develops into or is replaced by that peculiar wheezing note, characteristic of the latter part of nest-life, a sort of nasal whine, apparently made by expelling the air through the nose, and often with the bill closed. This note is given repeatedly and in chorus, at every visit of the old birds, or in response to any stimulus which arouses first one and then another nestling in quick succession, when the whole performance suggests the seething of a boiling pot or the hiss of escaping steam.

The power of orientation (4) is quickly established the moment the bird entirely quits the shell and becomes exposed to the full influence of the air. If the cuckoo is turned on its back, it will

struggle to right itself, and usually succeeds in doing so promptly. If this tendency is present while still within the egg, the power of independent movement must then be practically nil. Gulls, and probably many other species, turn their eggs in the nest, so that when pipped, the bill of the little bird lies uppermost.

As was pointed out in 1901, the huge pot-belly of the nestling of an altricious bird has its uses apart from the function of digestion, for as in the modern "brownie," it tends to keep the bird right side up. The pliant viscera, like water in a thin walled bag, conform to every movement, and form a central pedestal long before the legs can be used effectively for support. When the nestling stands, however, in this way on its abdominal mass, both the legs and rudimentary wings are used instinctively to steady it.

The newly hatched cuckoos lie flat in the nest with heads down, and sprawl in the same manner when taken in the hand, and laid on any flat surface, with claws clenched, and wings spread. The eyes commonly begin to open on the second day (table 6). When the nest is simply jarred, but otherwise undisturbed, the typical feeding reaction is usually given, whatever the age or degree of hunger, by all the nestlings simultaneously, or gradually, whenever the sounds and movements of one act upon the others in succession. Any vibration or sound whatsoever, whether due to a locomotive whistle, the calls of the parents, the notes of birds of other species passing by or to disturbances of the wind, produces the same reaction with more or less uniformity, and the older the bird, up to the seventh day, the more prompt and vigorous is this response. When one of the young behaves in this way, the others rise and respond to it in turn, precisely as to the parent. The young remaining in the nest will react to the call of one of their mates in the bush as freely as to the old bird approaching with food, and when a partly fledged bird, which I had held captive to prevent its escape, was returned to the nest, the other nestlings saluted it, and begged for food as they did of the parent. When it settled they put their heads under its wings, and tried to burrow under it, as if it were the old bird about to brood, and in their strenuous efforts for cover nearly turned it out of the nest.

9. THE QUILL STAGE

One of these birds in the complete quill stage (fig. 6) which is reached on the 6th or 7th day, was 111 millimeters ($4\frac{1}{2}$ inches) long, and weighed 31.68 grams (no. 1, table 2), in the space of 7 days having increased fourfold in weight and more than doubled in length. It now bristles like a porcupine in every feather-tract. The steel gray quills correspond in position, to the white 'hairs' which, as we have seen, they push out and for a time bear at their tips, like a short whip on a long stalk. Both in length and in diameter these tubes have increased many

TABLE 2

Rate of growth in the cuckoo

NO. OF EGG OR BIRD	DATE, 1908, AUGUST	1	2	3	4	5	6	7	8	9
1	Weight	7 56	11.16	16 20	21 24	25 2028	1631	68		
2		6 66*	6 66*	9 00	12 60	19 8022	6826	2828	0839	60
3		7 02*	7 02*	7.02*	7 02*	7 20				
1	Length of body (stretched)	54	64	73	83	90	100	111		
2		29×22*	29×22*	59	69	76	85	95	106	113
3		30×22*	30×22*	30×22*	30×22*	20				
1	Length, wing	15	20	25	31	32	38	43	43	45
2				17	23	28	31	36	43	45
3						17				
1	Length, tarsus	10	12		17	17	19	20		
2				12	14 5	15	17 5	19	20	22
3						10				
1	Length, long- est primary	5	5	7	11	16	20	25		
2				4	6	8	11 5	16	21	28
3						3 5				
1	Length, tail- quills	2	3.5	6	8	8	11	15		
2		0	0	1 5	2	3 4	6 5	9	13	16
3										

*Egg. Measurements in grams and millimeters.

†Disappeared from nest.

fold, the primaries from 5 to 25 millimeters in length (fig. 21, *i-j*), and the tubes of the dorsal tract from 8 to 20 mm. (including the flagellum of the primary down). Each quill tapers rather abruptly to its tip which is generally closed.

The feather-tubes of the linear abdominal tracts (fig. 21, *e-h*) which are commonly the first to burst, have a length of about 8 millimeters, and are yellowish white from the transparency of the tubular wall, and the color of the enclosed feathers. A few rudimentary down-hairs are still found on the back, and are later replaced by contour-feathers. The proper tail-quills which now appear as short thick, yellowish tubes, are not preceded by rudimentary down (fig. 21, *o*); the tail-coverts, however, are flagellated.

Towards the close of the completed quill stage the behavior of the young cuckoo undergoes marked changes. It indulges in new attitudes, acquires new alarm and call notes, shows fear, and begins the preening or combing movements, which later affect an apparently sudden and wonderful change in its appearance. In watching these birds closely from the tent, the first striking change is seen in their general attitude. They begin to sit up and take notice. The nest is no longer the center and bound of their visual world. They attend to all kinds of sounds, and respond to many of them, and while thus in the nest one will occasionally give the feeding reaction, thereby starting the others, who always react to the first bird; they will peck at crawling ants, or at mosquitoes, which have thitherto tormented them, unmolested; they notice the leaves and branches about their nest, when stirred by the wind; in short they begin to sit much like a brooding bird, with head upturned, and attend to everything. If the heat of the sun causes them discomfort, they move restlessly about the nest, gape, and pant with vibrating glottis, tongue and lower mandible, precisely like an old bird.

The preening instinct, in the course of a few hours from the first observed movement (in one case on the sixth day), becomes very active; with its bill the little bird proceeds to comb every quill within reach, quickly drawing its mandibles over it from base to apex (fig. 19). With apparent suddenness at the close

of the sixth day the horny sheaths of many of the quills begin to give way at their base, and are raked off by the mouthful, thus at one stroke exposing a few of the contour-feathers, which quickly fluff out to their normal proportions. In course of time these empty tubes come to litter the nest, and can be seen on the ground beneath it (fig. 21, *r*). Yet for this partly fledged bird, with senses becoming hourly more acute, its shallow platform of a nest is still for a time surrounded by an invisible wall, and no amount of heat or discomfort can drive it out. If handled at this time the scales and feather sheaths fall from it like ashes from a cigar.

To one who has not watched the course of events daily and hourly, this emergence of the true feathers seems both more sudden and more complete than it really is. Where before you saw a bird bristling with quills, after the lapse of twelve hours you find one clothed in soft feathers. The change is certainly striking, but it is neither sudden nor complete. As is well known, the feather-tubes of altricial birds commonly break away slowly from apex to base, in fine powdery scales, and the contour-feather is gradually exposed, passing from the tube or pin-feather, to the 'paint-brush' stage, and so on until the whole vane is clear. This is also true in some degree of the cuckoo, for at the climbing stage the tail and wing-feathers of flight are exposed only at the tips, their horny sheaths breaking away centripetally precisely as in other birds (fig. 21, *i-m*). At this time the primaries are freed for about one-half their length (figs. 4 and 8). This is also true of most of the feathers of the back (dorsal tracts), while many of the tubes of the head and throat, which are inaccessible to the 'comb,' are still intact. The sheaths are removed completely and entire only from the breast and sides (fig. 21, *r*).

Dugmore,²⁶ who was I believe the first to describe the apparently sudden emergence of the feathers in the yellow-billed cuckoo, says in one place that "The young when hatched are entirely naked," and in another, that the young which had been hatched in an interval of three days since he had seen their nest, were "naked

²⁶ Dugmore, A. Radcliffe. Bird-homes, pp. 6, 135. New York, 1900.

objects, with but the first beginnings of the pin-feathers showing, and that the feathers remained sheathed until the day before the birds left the nest." "Then in twenty-four hours *every* envelope burst, and the bird was completely feathered, with no trace of the sheathing except at the base of the tail." The "blue pin-feathers" of the down have evidently been confused with the feather-tubes of the 'Quill stage,' which in the yellow-bill is preceded by a dusky gray instead of a white rudimentary down, as I am informed by Dr. Ned Dearborn, who has studied this species. The process of unsheathing is probably a mixed, and for most of the feathers a gradual one, as we have shown to be the case with its close relative, *Coccygus erythrophthalmus*.

The power of association is far stronger in checking the feeding reaction than the influence of hunger in producing it. The effect of satiety, however, is seen when the stomach or gullet is full. Thus the last bird to be fed is frequently the only one not to respond upon the prompt return of a parent bearing food. Such a bird may be occupied in preening its feathers, while the excitement of its fellows has reached to the highest pitch. Again, either when satisfied with food, or when the stimulus is not of the right sort or intensity, the nestling will not give the food-reaction, but responds with a simple monosyllabic *cuck*.

During the last two days in nest, the young when undisturbed, give the food-response but seldom in the absence of the parents, when fed with the usual frequency. Thus on the fourth day of observation at nest No. 3, July 25, when all were in the quill stage, the feeding reaction was given spontaneously but once during a space of one and one-half hours, without the presence of the old birds. Meantime they would frequently respond to the call *koor-uck-uck-úk* of the distant parent in a note of similar quality. About the fifth day the young have developed a rolling guttural call, like *ker-r-r r-ék*, and this a day or two later, at the close of the quill stage seems to pass into the responsive *ker-ut-ut-út*, or *koor-uck-uck-úk*. Accordingly this note which originates as a responsive call is apparently modified later to serve as an alarm. Towards the close of nest-life the peculiar seething, pot-boiling sounds which these young utter when calling for food, or

when stirring about under the brooding parent as described above, are often interspersed with the responsive *ker-ut-ut út*, which then often sounds like *kar-ach-ách*, or when very hungry and excited they break into an explosive squeal like *kar-achít*.

When regularly fed, and otherwise undisturbed by the heat, their nest mates, or by loud sounds, the young lying flat, doze and sleep a good share of the time. The instinct to turn the head to one side and sleep with bill buried in the feathers of the back does not come until later, if at all. Up to the fifth or sixth day the chief exercise is derived from the grasping reflex, and the food response. Then with the preening or combing instinct, early in the seventh day, comes a new kind of exercise, associated with some pecking especially at crawling insects, the nest material, and occasionally at one another. The pecking is very indefinite at first, and I have never seen a case where a single insect was secured in this way either in these or other nestlings. Characteristic stretching of the wing and leg of one side is not seen in the nest-life of the cuckoo, because they leave the nest before prepared for flight, but they sometimes partly stretch one or both wings and duck the head before picking at the feather-tubes. Yawning has been noticed but very seldom, but the gaping and "panting" reflex, due to excessive heat, are as characteristic of the young at an early age as of the adult. The vibratory throat-movements of rapid respiration have not been seen, however, until the sixth or seventh day. When taken in the hand on the sixth day, while the instinct of fear is beginning to rise, the young will sometimes peck rather feebly as if in defense. Association with the nest was shown by a bird which on the seventh day climbed to a point a foot or more away and returned again, but this was observed but once.

The instinct of fear commonly matures at the beginning or close of the sixth day. When they are now closely or suddenly approached, a frightened nestling will sometimes clear the nest with one bound, seize hold of a branch and cling to it, and if it drops to the ground, it will make off with surprising speed. (fig. 10). When handled this young one utters a loud explosive squeal or danger-call, like *kar-r-r-r-r-éh*, or more like the adult *koor-uck-uck-úk*,

than which nothing seems to arouse the parent, and especially the female, to quicker attack or bolder measures. When touched, however gently, the little bird spreads its wings, stiffens, and lies flat with every quill and feather erect (fig. 11) and it repeats these defensive measures as often as it is touched or disturbed. After recovery from such a state it will bound out of its nest as often as it is returned, and for all such nest-life is at an end. (Compare p. 206.) If the nestling reaches the ground it is difficult to follow it, and even more difficult to recover the bird when once it has found any grass or shrubbery.

Under these circumstances the behavior of the old bird, especially if a female, is equally interesting. She is on the spot in a moment, and flies low, often coming close to the head of the intruder, giving a peculiar mewing sound, like a catbird, and an equally peculiar note, like *kek-kek-kek*, punctuated with the same high-pitched explosive alarm heard in the young, or flipping the wings, spreading and pumping the tail, breaks into the more ordinary alarm,—*Ker-ut-ut-út*, with the last syllable bitten off particularly sharp. With excitement at its height a brisk snapping of the mandibles is heard, while at a lull, the bird will stop to preen.

10. THE CLIMBING STAGE

With about half of its feathers freed in the way described, usually on the seventh or eighth day, the young cuckoo suddenly leaves its nest, and enters upon a climbing stage, (fig. 8) which lasts until flight or for at least fourteen days (table 6). The act is interesting, and since it has been repeatedly seen when performed under natural conditions, and by three birds in succession in one instance I will describe the procedure in the case of the oldest bird (table 6, bird no. 1, nest no. 3). With the sun shining full, but not excessively hot, this cuckoo crawled to the far side of its platform, combed off several mouthfuls of feather-tubes, and sat bolt upright; then directing its attention to a small branch, which rose from under the nest, it begun to duck and raise its head, much as an old bird might do when preparing for flight. Presently it

cleared the nest with a bound, caught a twig, and held on with head down (attitude shown in fig. 10). In a moment it had pulled itself up, and was comfortably perched.

This is the first step, and what happens next depends as much upon the actions of the old birds as upon the young. If no danger threatens, this bird is likely to be fed at the first perch, close to its nest, in the nesting tree, and if this spot is shaded and otherwise comfortable, association with feeding at this point will be quickly established, and may hold it there for a long time. Ordinarily however, the climber is liable to move about in the nest-tree, and if it drops to the ground will mount into the bushes again, and finally select a perch a few feet or yards away, to which through association it may hold for days, if undisturbed.

What actually happened in the case just described was different, for, having learned from experience the unpleasant consequences of losing even one of the nestlings owing to the division and diversion of parental attentions which inevitably follows, I immediately returned this one to its nest, but it promptly jumped out again. This was repeated three times in five minutes, when at last it dropped to the ground, was quickly enticed away by one of the old birds and was soon gone past recovery, although the grass and bushes were beaten in all directions. At another time I tried to hold the climbing young bird in an enclosure of wattled twigs, but to no avail, for they readily scale all such barriers, and I only succeeded in keeping them captive by taking them home with me for the night. It is only by thus securing and holding the climbing birds that observations on a nest of cuckoos can be conducted with any satisfaction, and prolonged for a period of eight or nine days, for with the successive withdrawals of the older young from the nest, the instincts of the parents seem to be satisfied by the attention bestowed upon those in the bush. In any case the last young, if backward in development, is sometimes left to starve, as I have noticed in one or two cases.²⁷

²⁷ The last bird in nest no. 1 was thus abandoned and several years ago I found a nest of the cuckoo with a young bird in the quill stage resting in it, and looking quite life-like. It proved to be a dried "mummy" and was probably deserted in the same manner.

In its climbing stage the young cuckoo profits by the strength with which it was born endowed, and the exercise which it has received through the grasping reflex, for it is a perfect acrobat, and there seems to be no necessary feat of climbing of which it is incapable. Like the young Hoatzin of the Amazons, it helps itself in climbing with its bill, though wanting the two free fingers of the wing, which in the South American bird are furnished with hooked claws. Though peculiar in many other respects, the Hoatzin, moreover, has been regarded by some authorities as related to the cuckoos.

When found occupying a natural perch the climbing cuckoo stands perfectly still and erect, with head upturned, suggesting the attitude already described for the brooding adult, when the nest is cautiously approached. When pressed too close it will drop to the ground, and disappear with a speed and agility, which would tax the resources of any animal not quick as a cat.

11. RECORDS OF NEST-LIFE AND BEHAVIOR

In the section on cyclical instincts we have included under 'care of the young' (term 6, table 1), a variety of highly complex, but related actions of the lock and key order, which involve both parent and child. When behavior is known to be perfectly free, as it was on the second day of observation of either nest, number 1 or 3 (tables 4 and 5), it appears to be stereotyped or as nearly uniform as we have a right to expect in animals so intelligent as birds. No activities of the complexity here shown are ever perfectly uniform, but before considering the factors which modify behavior under this head, we shall consider the food habits of both adult and young.

A. Food of adult cuckoos

The food of the black and yellow-billed cuckoos has been studied by Beal,²⁸ whose results were based on an examination of 25

²⁸ Beal, F. E. L. Cuckoos and shrikes in their relation to agriculture. Bull. U. S. Dept. of Agriculture, no. 2. Washington, 1898.

stomachs of adults of both species, gathered from a wide area, and chiefly between May and October. Their diet was found to consist almost exclusively of insects, and in great variety, one stomach only containing vegetable food, and this but two berries of the rough-leaved cornel, all other vegetable débris coming from the great numbers of caterpillars of lepidoptera which devour vegetable tissue.

Much has been written to show that the lapse of the brooding instinct in *Cuculus canorus*, and even the small size of its eggs are due to the innutritious character of its food. Thus it has been maintained that their ordinary caterpillar-diet contained so little nourishment that it was necessarily taken in great quantities, with the result of distending the stomach, imperfectly nourishing the growing eggs, and leaving no time for the process of incubation. It is unnecessary to dwell upon the absurdity of these statements, but this is probably the first time that reduction in the size of the egg, or abbreviation in development, has been directly attributed to the nature of the food. The kind of reasoning employed by advocates of this theory is fallacious at every point, and is contradicted not only by the fact that many brooding species live like the non-brooding European cuckoo on a miscellaneous insect diet, but by the more significant circumstance than the American cuckoos eat the same kind of food, and yet lay large eggs, and rear their own young.

B. Food of young cuckoos

When the supply is abundant, birds as a rule, feed their young such food as in their habitual methods of search they find and eat themselves. The food of nestling cuckoos is illustrated in tables 3-5, in which the daily fare is shown to consist, like that of the adults, of a miscellaneous assortment of insects, no fruit whatever having been served at their nests. Smooth larvæ represented 44 per cent of the total food, and hairy caterpillars and adult lepidoptera about 5 per cent each, while grasshoppers, which Beal found to constitute 30 per cent of the adult pabulum, furnished 27 per cent in the case of these young. The smooth larvæ were

mostly brown, green or gray, and from one to one and one-half inches long representing small lepidoptera, and possibly crane flies, while the larger, from two to three inches in length, were without doubt larvæ of some of the sphinges, and larger moths. The hairy caterpillars were apparently those of common butterflies, such as *Vanessa antiopa*, and of various small moths. The adult lepidoptera were white and gray moths, some having the size and appearance of the brown-tail. I have never seen a single adult butterfly served at the nests of this cuckoo.

TABLE 3

Food of nestling cuckoos, nests nos. 1-3.

NO. OF DAY	1	2	3	4	5	6	TOTALS	PER CENT
Smooth larvæ	4	14	24	10	1	1	5	44
Grasshoppers.	5	1	3	7	12	5	33	27
Caterpillars	1	5	0	1	0	0	7	5 6
Moths	2	0	1	1	2	1	7	5 6
Other insects	1	5	5	6	1	4	22	18

No. of nestlings: 6.

Total no. of visits with food: 123. Total time: 53 hrs. 4 min.

Highest average daily rate of feedings, once every 8.6 min. Total average rate, once every 26.3 min.

C. Method of feeding young

The young cuckoos are fed by both parents whether in the nest or out of it, but the instincts of the male in this direction are much the weaker, and are liable to be checked by fear. At nest no. 3 (table no. 5) I have recorded 13 visits by the male, but as the sexes were indistinguishable in size or color, and could be judged only by their general behavior, these records are not very trustworthy. After the first young have climbed out, the male seldom, if ever, visited the nest, but gave his attention to the bird in the bush.

Cuckoos often seek their food a long way from the nest-site, and while both parents are occasionally absent at the same time, I did not often find this to be the case in the early days of nest-life. While engaged in the search the responsive *kow-kow* is frequently indulged in, and this often proved the signal for

approach, the notes becoming louder and louder as the old bird neared the nest. When within a rod or but a few yards from their young, these birds frequently come and depart in silence if their behavior is free, but whenever fear enters as a disturbing factor every approach is slow and cautious in the extreme, the bird stealthily flitting from bush to bush with food in bill, flipping the wings, and pumping head and tail in their characteristic manner making long pauses at one place, and sometimes encircling the nest more than once before actually entering it. Again when no disturbing influences seem to be at work, a very low and peculiar mewling sound, like *kar-r-r-r-*, is heard close by the nest, and this acts both as a stimulus to the young, and as an admonition to the adult, for the young respond eagerly, especially when this is repeated at the nest itself. If uttered by the approaching male, when the female broods, it is so well understood, that although unable to see her mate, she promptly leaves the nest, takes the insect which he bears, and returning quickly feeds young, inspects, and settles down to brood again, or if the nest is cleaned, leaves, and later returns with food. If the timid male approaches but fails to give the admonitory note, the female soon perceives him utters a somewhat similar low crooning note, like *wur-oo-oo*, and acts promptly as before. Still again, when the nest is reached, and the young are quiescent or respond but feebly, this peculiar note is given, and serving as a 'tickler' or 'teaser', never fails to arouse the nestlings to the highest pitch of excitement of which they are capable.

The final approach to the nest is made quickly, and along a definite path, which has been determined by habit or association, as I have shown to be quite generally the case with birds, whenever their behavior is not distracted by fear. They go to a certain tree or bush, to a certain branch, grasp certain twigs, and finally enter the nest on a certain side, or in a certain manner.

The nestlings, if not previously aroused by the sound or sight of their parent, or by the vibration to which the nest is subject, are stimulated by the final appeal just described, and the 'pot-boils', that is, each one rising on its pillar-like abdomen, or later on its toes, with upstretched and trembling neck and vibrating

TABLE 4
Activity records of cuckoo at nest no. 1

DAY OF OBSERVATION	1		2		3		4		5	
	27		28		29		30		31	
DATE, 1908, JULY										
TIME	A. M.	P. M.	A. M.	P. M.	A. M.	P. M.	A. M.	P. M.	A. M.	P. M.
10 10-12 35 3 Hrs. 25 M.		2 40-5 28 3 Hrs. 48 M.	9 30-12 17 2 Hrs. 47 M.	2 25-5 00 3 Hrs. 35 M.	9 50-12 20 2 Hrs. 30 M.	2 45-4 35 1 Hr. 50 M.	9 40-12 35 2 Hrs. 55 M.	3 20-6 35 3 Hrs. 15 M.	8 45-1 50 5 Hrs. 5 M.	
WEATHER-CONDITIONS	Fair	Cloudy	Cloudy	Cloudy	Cloudy and humid	Fair, hot and humid	Fair, hot and humid	Cloudy	Fair, hot and very hot	
Number of birds in nest...	3	3	3	3	2-1	1	1	1	1	
Number of birds in bush	1	1	1	1	0-2	2	2	2	3	
Number of birds in captivity										
Total number of feedings		4	7	10	1	1	1	1	1	
Visits without food	0	0	0	0	0	0	3	4	1	
Visits by ♂ identified	1	1	1	2	0	0	0	0	0	
Average frequency of feedings in minutes		27	15	21	75		58	49		
Food.										
Hairy caterpillars		3	3	2			1	2		
Smooth larvae		3	3	7	2		2	1	1	
Grasshoppers	2*	1	1	1			2	1		
Other insects								1		
Inspection and Clean- ing			2	1	1			3		
Excreta moved										
Excreta eaten	1	2	2	7			1	1	1	
Brooding bird				♀						
Brooding				1						
Shielding				38			1			

*Brought to nest but not delivered.

TABLE 5

Activity records of cuckoo at nest no. 3

DAY OF OBSERVATION	1		2		3		4		5		6	
	22		23		24		25		26		27	
TIME	A. M.	P. M.	A. M.	P. M.	A. M.	P. M.	A. M.	P. M.	A. M.	P. M.	A. M.	P. M.
	3:30-5:40 2 H. 10 M.	10-12:52 2 H. 52 M.	3:08-5:35 2 H. 27 M.	3:08-5:35 2 H. 27 M.	10:10-12:50 2 H. 40 M.	3:15-5:45 2 H.	10:30-1:45 3 $\frac{1}{2}$ H.	9:05-12:30 3 $\frac{1}{2}$ H.	3:30-5:10 1 H. 40 M.	9:10-12:15 3 H. 5 M.		
WEATHER CONDITIONS	Fair		Cloudy and wind		Rain		Rain, variable		Fair and hot		Fair and hot	
Number of birds in nest	3		3		3		3		3-2		2	
Number of birds in bush					1		1		1-2		2	
Number of birds in captivity												
Total number of feedings	6		5		3		24		12		5	
Visits without food	0		1		0		3		2		0	
Visits by ♂ identified	1		1		1		6		2		1	
Average frequency of feedings in minutes	11		22		29		4		7		5	
Hairy caterpillars	1								10.8		7.5	
Smooth larvæ	1		3		1		16		6		1	
Grasshoppers	2		1		1				3		4	
Other insects	3		2		1		5		1		6	
Inspection			2		2		4		3		6	
Excreta removed and cleaning	4		2		3		8		3		1	
Excreta eaten											2	
Number of times	5		3		3		9		4		1	
Time in minutes	22:15		9:8		5+ :98		5+ :15:1:12		12:15		13	
Brooding	17:8:10		28		17		4:15:4		25+		25+	
Brooding bird	♀		♀		♀		♀		♀		♀	
Shielding, times and minutes					6:3+				1:24			

+ Indicates that bird was disturbed while brooding.

wings, opens its mandibles to the limit, and utters the wheezing grating note characteristic of infancy (figs. 14 and 15). The parent having landed on the nest-wall with grasshopper, hairy caterpillar, or large green larva, pinched usually just back of the head, and hanging limp from the bruising process to which it has been subjected, sometimes simply places the tip of its bill in the mouth of the young, or lays the free end of the insect across the mouth-opening (fig. 13), and holds it there. The mandibles of the nestling close, but no swallowing effort is apparent for some time. Thus interlocked, parent and child will sometimes remain as motionless as statuettes for five minutes by the watch, and it is quite common for them to hold to this position for one or two minutes. Then the mechanism in the throat of the young bird begins to work, the swallowing reflex is started and the insect gradually slips down as if greased.

When an old bird with food in bill is startled by some unusual sound or thoroughly frightened in any way, it first swallows the insect before giving any alarm, or taking any measures to protect itself. The persistence, however, with which they will endeavor to feed their young was shown more than once when the great size or some other peculiarity of the insect carried made it unmistakable. Thus on one occasion the female brought in a bright green larva, not less than three inches long, and of about the thickness of a finger. It was offered to the young both in the bush and at the nest, but in vain. This great larva was gripped behind the head, and was thus carried about for the space of half an hour, when it was brought to the nest for the second time, and again the bill holding the insect's head, was placed in the mouth of one of the nestlings, the long body of the larva hanging free at one side. It was on this occasion that five minutes were required to awaken the swallowing reflex.

While in such cases as that described the immobility of parent and child is most marked, the mother will sometimes remove the insect and replace it, or withdraw it a little way as if to give it a fresh start, or repeatedly lift it up and down ten times or more while awaiting the proper reaction. Under these conditions the mouth of the nestling is watered by a copious flow of saliva. On

still another occasion at the same nest, when a large grasshopper was offered it was placed in the mouth of the same bird and withdrawn 21 times in succession, before it seemed to strike the right spot, or at least to produce the proper response and to be quickly swallowed. It very rarely happened that the swallowing reflex followed promptly when the food was simply inserted in the mouth.

So novel was this striking performance, and so commonly was it observed at nest no. 1, it seemed as if it were the general practice with this cuckoo, but at nest no. 3 the food was more commonly placed at once deep down in the throat (figs. 14, 15), where every trial was a reaction-test, and upon failure to swallow promptly, the food was withdrawn and another nestling was tested, precisely as in vireos, thrushes, and other passerine birds. This variation of the feeding process, which is remarkably uniform in the species previously studied, is one of the most interesting facts observed, in the nest-life of this cuckoo. I have received the impression, without certainty of its being correct, that the more nearly free the behavior, the more commonly is the method of mouth-feeding resorted to.

Minor variations in the act of serving the food, some of which are referred to above, are often very interesting, and seem to clearly imply intelligence by the way in which means are quickly adapted to the end evidently sought. Thus, on one occasion the mother offered a large grasshopper to bird no. 2, withdrew it when there was no response, and placed it in mouth no. 3. When this bird had sat panting with insect in bill for half a minute, unable to swallow, the female again withdrew it, and with the rapid movements of her mandibles minced it so fine, that when no. 3 was tried again, it slipped down quickly.

Again at the last day of observation at nest no. 3, the female brought a grasshopper, and in trying to land it in the mouth of one of the young, dropped it into the nest and went off as if frightened. In twelve minutes she returned empty-handed, looked for the grasshopper, picked it up, but in failing to deliver it, bore it away. In ten minutes she was back again with what looked like the same insect, and after trying unsuccessfully to serve it for the third time again carried it away. This illustrates their econ-

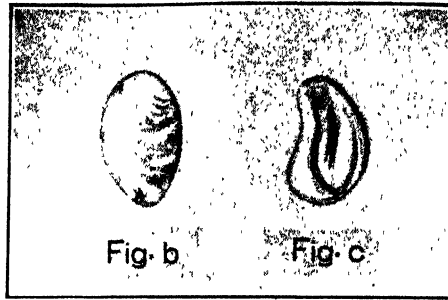


FIG. *b* Excretory sac of cuckoo, muted four hours after birth, consisting of elastic mucous wall, and semi-liquid contents; the dark portions represent the indigestible remains of insect-food. Natural size.

FIG. *c* The same sac after being rolled, showing elasticity of unbroken wall. Natural size.

omy in food, as in the case of the larva given above, even if the question of memory be left in doubt.

D. Inspection and cleaning in cuckoo

When the food has been taken, the parent pauses, and stands at inspection. With head slightly depressed, she scrutinizes the nest, and watches the bird which has just taken food. This nestling soon betrays uneasiness, and with raised wings quickly tilts the hinder end of its body upward and toward the outer margin of the nest-platform. The white sac, as it leaves the cloaca is instantly seized by the old bird, and either eaten or carried away (fig. 12) its disposition depending upon various circumstances, in which, as I have elsewhere shown, must²⁹ be included the hunger of the old bird at the moment. The condition of the nest and the behavior of the other nestlings also help to determine the act. Attention to the nest itself usually follows that given to the young and the parent will commonly walk around the nest to pick up any sac which may have fallen on its surface. That the excreta in these cuckoos are much more frequently eaten than removed is shown by reference to tables 4 and 5, where in 60 recorded cases, the excreta were devoured 38 times, and removed 22 times. When the sac is removed, the bird flying low with depressed head,

²⁹ Home life of wild birds, p. 191.

bears it stealthily away, and probably drops it at some distance from the nest, but owing to the wooded character of their surroundings this could not be determined. Toward the close of nest-life, the timidity of the parents seem to rise, and the cleaning instinct certainly wanes. Sacs are then allowed to remain on the nest, which at an earlier period would not have been tolerated for a moment (see fig. 11). This characteristic has been often noticed in other species.

The rapidity with which the process of digestion proceeds in the young bird is remarkable. The sac of a nestling cuckoo which hatched four hours after birth (figs. *b* and *c*), already contained the residue of digested insect-food. This was a small elastic, transparent bag, with tenaceous wall closed on all sides, and filled with fluid and solid substances. It was odorless, and like a rubber water-bottle could be picked up and rolled about without breaking or soiling the fingers. When punctured a clear liquid came out, leaving a milk-white residue, with streaks of dark masses, more or less involved in the mucous, and composed of the hairs, scales and chitinous fragments of insects. The milk-white substance, which dries to an impalpable powder, is not affected by acids or chloroform, but has the appearance of an emulsion being composed of very fine globules.

The removal of egg-shells or of addled or broken eggs is probably to be referred to the cleaning instinct, and in some species it is promptly and almost invariably performed. When an egg hatches in the nest of the blackbill, the empty shell is not as a rule, immediately removed (table 6), the instinct to brood being apparently stronger than that to clean the nest. They are seldom allowed to remain very long, but the shells of the last egg to hatch are apt to be entirely neglected.

E. Rate of feeding young

When behavior is normal, in those species whose young remain in the nest until all are ready to leave, the rate of feeding tends to increase from the time of hatching until it reaches a certain average, to which it may hold for many days with surprising

constancy. With an abundant food-supply the rate of feeding is determined to a considerable degree by the weather, and seems to be most rapid on hot clear days. In very bad weather, as in heavy rains, feeding stops entirely, when it is replaced by brooding, which in one recorded instance (table 5) lasted in this cuckoo, one hour, and thirty-eight minutes.

The rate of feeding in 125 observed cases at nests Nos. 1 and 3 covering a period of 53 hours, was once every 25 minutes, but this is undoubtedly below the normal rate, and such figures have no significance unless the variables are known. At nest no. 1 the rate of feeding began to fall the moment the first bird escaped to the bush. The highest feeding rate for an entire day (table 5, third day,) once every 4 minutes, is of more interest, since such frequency is only observed in this cuckoo when fear is in abeyance, food abundant, weather favorable, and there are no young in the bush to divert the attentions of the parents from those at the nest.

F. Intermittent brooding and behavior of cuckoo in rain

The regular brooding instinct, which begins when there are eggs, and reaches a climax shortly after the young are born, is commonly on the wane when the nestlings have reached the age of two or three days, and seems to shade off into what I shall call "intermittent brooding." In the cuckoo from shortly after hatching to the age of one week, the young are brooded intermittently, subject in all probability to changes of temperature and light-intensity. When excessively hot or humid, this seems to shift into the shielding or spreading reflex. Thus a bird which begins to brood in great heat, will often gradually spread her wings, and assume the typical attitude, sometimes standing erect instead of sitting on the nest. Without any doubt the young are brooded continuously at night, but how long this night-brooding lasts I was unable to determine. While intermittent brooding is evidently related to the more continuous brooding of an earlier period, there is this difference that fear is again in the ascendant. How far intelligence enters into the question of brooding, as it possibly does, I am unable to determine.

Observations on the brooding activities of birds in the wild state are so meager, it may be worth while to give such as can now be offered in some detail. The female at nest No. 1 (table 4) brooded but once during the four days of our observations, but then for a period of thirty-eight minutes, beginning at 4.45 p.m.; the weather was cool and cloudy, and the youngest bird in the nest was four days old. At this visit the old bird, after feeding her young, raised her wings slightly and settled over them, repeatedly erecting and dropping her breast-feathers, to let the nestlings under and to cover them. With head-feathers often erect, occasionally turning her head from side to side, the bird sat quietly, and was keenly alert to every sound and movement about her, rising only to accommodate her strenuous young, which seemed never to rest, but were constantly burrowing about, uttering their low grating notes in chorus, and poking out their heads.

In illustration of habit we may notice here that brooding birds almost invariably face the same way (compare figs. 17-18), the habit being formed quickly, and held to persistently. They face the side of easiest approach, which also commands the widest range of vision. When the tent was erected beside the nest on the first day, it was three hours before this bird would return to her young; on the second day behavior was unrestrained, and she brooded for over half an hour within reach of the hand. All the nests studied were in the same pasture, and cows with their clanging bells often brushed close to tent and sitting bird, but such sounds had become familiar, and to either parent, whether engaged in brooding or feeding its young, they offered no terrors.

Since nest no. 3 was too low for convenient observation, the entire tree was cut off, raised six inches, rotated to avoid obstructing branches, and secured by its base in a well, sunk in a stake, which was in turn set deep into the ground. When this nest was closely approached for the purpose, the male who happened to be coming with food, gave the staccato alarm, *koor-uck-uck-úk* ate his insect, and wiped his bill, but the female maintained her position until driven off showing that this alarm is not necessarily effective when its source can be seen. The female after leaving gave the same call, and the male continued to repeat it

during the forty-two minutes that operations at the nest lasted. At 3. 30 p. m. two hours and forty-five minutes later, when the place was next visited, the female was brooding on the displaced nest, with her head turned in the same direction as previously noted. When frightened off this time, observe that in twenty minutes after the tent was entered, she captured and brought a large insect to the nest, held it four minutes in the mouth of one of the three nestlings present, until it was swallowed, inspected her brood and picked them all over, then settled over them and remained 22 minutes, until the call of the approaching male served as a signal for her relief. These details are given not only to show the rapidity of habit-formation, but to prove how quickly the behavior of a timid animal may become free in the face of abrupt changes in its environment.

This nest still contained a single egg (fig. 15), which afterwards proved to be addle and was thrown out by the bird four days later. In entering the nest to brood, the cuckoo would clasp this egg with one foot, and after standing on it thus held for a few minutes, she would gradually settle down and draw it under her. In this way the egg was sometimes moved from the front to the far side of the nest. Gradually it came to be neglected, and can be seen to one side of the sitter in plate 6.

At times the length of each brooding period would depend on the activity of the male, for he had a habit of coming to within a few feet of the nest, calling out the female, who would regularly take the insect from his bill, return and serve it. She would then either remain to brood, or quickly depart and reappear with an insect of her own capture. The young often rise up in the same eager excitement when the parent departs, as when she comes to them bearing food, which may be cited as one of many indications of that lack of discrimination which they commonly show.

At nest no. 3 also, I had the opportunity to watch the behavior of these birds during two severe rain storms, the first and heaviest of which was on July 23 (table 5). In the first instance the rain began to come in torrents at 3.30 p. m., and almost at the same moment the female cuckoo was at the nest, fed her young, attended to their sanitary needs, and began to brood. As the water reached her, every feather at times became erect, when she

seemed to swell to a great size, and would then settle down low, and with partly spread wings attempt to shield her young from the storm. The water stood in beads all over her head, back, wings, and tail, and rolled off without wetting any of her feathers for some time. Gradually however the fine feathers about the bill, and eventually those of the head and throat became soaked, representing possibly those parts least accessible to oil, which is applied with the bill. The young continued their restless movements during the storm, as well as their chorus of hissing sounds. The rain lasted about one and one-half hours, and the brooding period one hour and thirty-eight minutes, during which the brooder was silent, and never shook off a drop of water. Two or three times she rose and examined her young, and would occasionally turn her head from side to side, open and close her bill, half close her eyes, or move to accommodate her strenuous nestlings. At 5.05 p. m. when the rain had about ceased a distant *kow-kow* note was heard from the male, and presently he approached, thoroughly drenched, and bearing a large grasshopper. At the sound of his low mewing note when a few feet away, but still out of sight of the sitter, the female slipped off quietly and disappeared. The male then went through the stereotyped round of feeding, inspection, cleaning, and in his turn retired. After an absence of eight minutes, the female again returned at 5.18 p. m., served her young, and resumed her brooding. The storm was then nearly past, and she had become wetter in seeking the food, than in sitting at the nest. Her respirations had fallen from 100 at 3.30 p. m. to 80 at this hour of the evening. Owing to her quiet attitudes it was possible to photograph her at any time during lulls in the storm by giving the plate an exposure of several seconds.

From the facts given above it would appear that intermittent brooding, which may continue for a week, after the young are hatched must be considered as a reaction to an external stimulus, in which temperature and other weather conditions play the most important part, but determined also in some degree by contact and visual stimuli, so long as there are eggs. In the earlier period of incubation the sight and touch of the eggs probably furnished the external stimuli for those profound changes in disposition and

general behavior, which we have elsewhere described as the 'rise of the brooding instinct.'

The activities of nest-life do not invariably follow in the single groove already described, for while the parents seldom visit their nest when it contains young, during daylight, without bringing food, they are quickly drawn to it by the presence of enemies, and occasionally merely to inspect or to brood. This was noticed only at nest no. 3, where out of 100 recorded visits by both birds, but 6 were made without food (table 5). In each case the female, and usually when brooding, would leave the nest quickly, presumably to forage on her own account, return, inspect, cleaning the nest if necessary, and then settle to brood.

12. "WHY SOME BIRDS DO NOT BROOD," OR ORIGIN OF "PARASITIC HABIT"

Although the question is often cautiously put in the form given above, it might better read, "Why have certain cuckoos and cowbirds lost their nesting instincts," for it is perfectly safe to assume that every one of these birds is descended from ancestors which at one time built nests, and brooded like the majority of their kind.

If, as Baldamus and others assert, the eggs of *Cuculus canorus* are laid at intervals of 6 or 7 days, it is evident that the successful rearing of young in this species would now be difficult if not impossible. It is equally clear that this unduly lengthened interval is a secondary condition, to which it is impossible to assign the lapse of the instincts to care for the young, as shown by the habits of our own cuckoos. The primary step which finally led to parasitism is to be sought farther back, and lay, as we believe, in that very lack of attunement between egg laying and nest building, to which all birds are casually subject, but which is especially characteristic of those cuckoos, cowbirds, and hangnests, among which alone in the great avian class this much discussed parasitism has been chiefly developed.

In the analysis of the cyclical instincts (section 5), we have seen that the reproductive cycle of birds is characterized by a series of discrete types of action, which may or may not be recurrent, and which follow in chain-like sequence, one kind of action being performed as if in anticipation of that which is to follow. Thus

the nest seems to be built in anticipation of the eggs which are afterwards laid in it, and these eggs to be guarded and treated in view of the young which are to issue from them. The normal rhythms are not only very precise and uniform but the correlated instincts of parent and child fit like key and lock. Yet perturbations, as we have seen, are liable to arise at every step, whether it be in migration, nest-building, egg-laying time or interval, or in the nurture of the young, and are more or less fatal according to circumstances. The young, moreover, sometimes fail in the development of their instincts, as when a thrush or warbler flees its nest before ready for flight, in consequence of the premature development of the sense of fear.

The most common failure is, without doubt, in the adjustment of nest-building to the time of egg-laying, and I believe that at this point "parasitism," as it is somewhat ambiguously called, in birds, took its rise. In many of these cuckoos and cowbirds there was a tendency then as now, not only to lay the eggs before there was a nest to receive them, but to lay them at slightly irregular intervals.

Dropping eggs on the ground and taking no further interest in them is a form of behavior, which if generally indulged in would soon settle the question of survival most effectively, and although this rigorous form of selection is only sporadic in most species, it is reported to be common in the European cuckoo, for many of the cowbirds, and probably occurs more frequently in all birds than is commonly supposed. According to Hudson, whose work on the cowbirds of Argentina has been frequently quoted, the common species of that country (*Molothrus bonariensis*) frequently waste their eggs by dropping them on the ground, and they even laid in the old nests which he placed in trees for the purpose to testing them. While this species is now generally parasitic, it scatters its eggs in all directions, and on two or three occasions was observed by this naturalist to attempt to build a nest of its own, but without success. The North American cowbird (*Molothrus pecoris*) is also known to occasionally drop an egg on the ground, and when it is realized that a fresh egg is a prize to every predaceous animal which roams by day or night, it is not surprising that such habits are not more frequently reported.

The door is thus opened wide to parasitism in its initial stage, whenever the acceleration of egg-laying or the retardation of the building instinct becomes common, with or without irregularity in the egg-laying intervals. A later stage in the retrograde direction is seen in the present-day habits of the common Argentine cowbird referred to above, which will lay in any kind of a nest, wastes its eggs, and though sometimes attempting to build is never known to rear its young. The visual stimulus produced by a nest seems to act mechanically upon this bird, much as the sight of an egg, although it be an artificial one placed in her nest, affects the female noddy tern. Watson⁸⁰ found that by placing such an egg in the nest of a "laying" noddy, he could change its habits from those of a "layer" into those of a "sitter." The mere sight and touch of such an egg seemed immediately to evoke the guarding and fighting instincts while the behavior of a "sitter" was reversed if her egg was removed. The production of a large number of eggs would seem to have saved this Argentine starling from utter extinction. The North American cowbirds without doubt, have passed along a similar path, and if they once squandered their eggs in the same fashion, this practice has been eliminated with the greater precision of their newly developed instincts.

The cuckoo of Europe has retrograded into parasitism through a course essentially similar, by the diversion of some of its own instincts into slightly different channels, and by the acquisition of new ones in both adult and young. Thus we find this cuckoo today frequently dropping its eggs on the ground, and when it does not abandon them, carrying them in bill to the nest of a nurse. This, as already remarked, must be regarded as one of the most striking instinctive performances of this bird at the present time. Fortunately we can point to a quite similar act in the black-billed cuckoo of the New World, for, as we have seen, when this bird's nest is disturbed, it has been known to remove its eggs to another nest, and to continue to care for them. It seems very probable that this removal of the egg in bill, of which instances could be given in other species of birds, is the survival of a very primitive instinct, and is possibly related to the old instincts of

⁸⁰ Watson, John B. The behavior of noddy and sooty terns. Pub. 103, Carnegie Institution of Washington, p. 223. Washington, 1909.

guarding and concealment, out of which the instinct of incubation, has, in my opinion, grown. Without any doubt the act is as purely instinctive as any other by which the ends of reproduction are secured to the species.

Parasitism could never succeed as a general practice on a large scale, and the fact that it is a specialty of two families of birds, shows that it is probably correlated with a peculiarity which they possess in common. This is to be found in a change in the rhythms of the reproductive activities, leading to a change of instincts. This change in the American cuckoos is now chiefly expressed in the appearance of eggs at various intervals, or at intervals greater than one day and rarely exceeding two. The disturbance has in this case been completely met and adjusted by the development of a climbing stage in consequence of which the young can leave the nest in succession, and nest-life can proceed with eggs and young existing at the same time with prejudice to neither. As to the "why" of this problem, that is, why has the normal rhythm of the reproductive cycle been disturbed in the way described, nothing is certainly known; we can only surmise that the causes of all such changes concern the central nervous system, and that in the main they are independent of food-habits.

The nests of these American cuckoos are slight, but adequate, and afford no more evidence of a decay of the nesting instincts than does the even cruder nest of the mourning dove, or many another wild species. However, in support of Darwin's views, it has been often pointed out that the yellow and black-billed cuckoos occasionally interchange eggs, and steal the nests of other birds. This is true, but since any bird is likely to steal a nest whenever its reproductive rhythms are upset at a certain point, it has no significance, unless it can be shown that such cuckoos as do appropriate nests, do not subsequently make nests or rear their own young. I have found recorded in the literature, so far as I have been able to review it (table 7) 24 cases in which these cuckoos have either interchanged eggs, or laid in other birds' nests. One of the most interesting observations under this head is quoted by Bendire²¹ from Mr. J. L. Davison, of Lockport,

²¹ *Op. cit.*

TABLE 7

Record of American cuckoos' eggs laid in foreign nests

CASES	NEST OWNERS	INTRUDERS	NO. OF INTRUSIVE EGGS	REMARKS
1	Yellow-billed cuckoo	Black-bill	1	{ cuckoo species not determined
1	Cedarbird	Black-bill	1	
1	Cardinal	Black-bill (?)	2	
2	Yellow warbler	Black-bill	1:1	
2	Chipping sparrow	Black-bill	1:1	{ Owner laid 1 egg, and the intruders 2 each
1	Wood pewee	Black-bill	1	
1	Robin	Black-bill Mourning dove.	4	
1	Catbird	Black-bill	1	
3	Catbird	Yellow-bill	1:1:1	
3	Robin	Yellow-bill	1:1:2	
3	Black-billed cuckoo	Yellow-bill	1:1:1	
1	Wood thrush	Yellow-bill	1	
1	Dickcissel	Yellow-bill	1	
1	Black-throated sparrow	Yellow-bill	1	
1	Cardinal	Yellow-bill	1	
1	Mourning dove	Yellow-bill	1	

N. Y., in which the latter speaks of finding three species of birds using the same nest. After stating that he had often found the eggs of the black-bill in the nests of the yellow-billed cuckoo, but in those of no other species but once, he gives the following account: "June 17, 1882, I found a black-billed cuckoo, and a mourning dove sitting on a robin's nest together. The cuckoo was the first to leave the nest. On securing this I found it contained two eggs of the cuckoo, two of the mourning dove, and one robin's egg. The robin had not quite finished the nest when the cuckoo took possession of it and filled it nearly full of rootlets; but the robin got in and laid one egg. Incubation had commenced in the robin and cuckoo eggs, but not in the mourning dove's egg. I have the nest and eggs in my collection." It is a pity that this remarkable struggle of instincts was not allowed to reach a conclusion. The case, however is one of exceptional interest to the student of animal behavior, but in interpreting it we must be careful not to be misled. It seems to give us a picture of one of the early steps through which parasitism must have passed, perhaps

we might say two of the stages, for observe that this is really a double structure. The cuckoo seized this robin's nest while still incomplete, and filled it with rootlets, that is, used it as a site on which to erect a nest of its own. The robin held its ground long enough to deposit an egg. Then came the dove and stole the nest from the cuckoo, laid her eggs in it, without adding any new materials, so far as known, and was trying to maintain herself in possession when discovered. That the dove came last is shown by the fact that her eggs were the only ones in which incubation had not advanced. The addition of the rootlets by the cuckoo shows how firmly instinct held the reins of action, and the visit of the dove, famed for her strong parental instincts, warns us of the folly of trying to fasten an incipient parasitic habit upon the cuckoo from such casual or sporadic acts alone. The fact that such a bird as the mourning dove, should steal a nest, and hold to it for the purpose of rearing its young, shows that failure to build a nest was without doubt due to a disturbance of the normal reproductive cycle, the eggs being forthcoming before the usual nest was ready. Under such circumstances it would seem that any nest provides a visual stimulus to the act of laying in it, as in the case of the common cowbird of Argentina. In a similar manner the brown thrush, quail, and many other species of birds have been known to casually lay eggs in the nests of other birds. Probably in many such cases and in the others given in table 7, as in the known instance quoted above, the intruder is ready to fight for possession, but commonly yields, and later builds a nest of its own.

The development of the parasitic habit of the European cuckoo, has led to a gradual lengthening of the egg-laying interval, and its young has acquired the evicting reaction, together with other secondary changes. Finally, to bring this long section to a close, we have only to refer to the African and South American ostriches, in which the instincts of the female have undergone most pronounced changes, leading to a great waste of their eggs, which Darwin, Schillings, and other travellers speak of finding freely scattered over the plains. The adjustment seems to have been effected here by the strengthening of the parental instincts of the male, upon whom almost the entire duty of incubation and care of the young is said to fall. Moreover the neglected eggs are

not wholly wasted, for those strewn about the vicinity of the nests are said to be largely devoured by the young.

13. CONCLUSIONS

1. Cuckoos do not display more intelligence than many other species of birds, the extraordinary acts which many of them perform being sufficiently accounted for by the possession of modified and highly specialized instincts.

2. The origin of the parasitism in many of the old World cuckoos and American cowbirds is to be sought in the disturbance of the cyclical instincts, to which it has been shown that these families of birds, are especially subject and in particular in the attunement of egg-laying to nest-building. Sporadic cases of this sort occur in all birds, when they either drop their eggs on the ground and eventually abandon them, or lay in other birds' nests, when they will sometimes fight for possession. We may assume that through the action of inheritance and selection the practice has become established more or less completely in the present parasitic species, but while we can indicate the steps of the process, the causes which have led to each in succession, can only be surmised.

3. American black- and yellow-bill cuckoos show a tendency to produce eggs at irregular intervals of one to two or three days, which accounts for the presence of eggs and young in their nests for a longer time than is usual, but here the comparison ends. Any disadvantage which might arise from such a condition has been completely allayed by an early division of the young, each one of which (in the black-bill) leaves the nest in succession on the seventh day from birth, and spends about two weeks in a climbing stage preparatory for flight. Special powers and instincts have arisen in the young in adaptation to this condition.

4. The evicting instinct of certain Old World cuckoos has apparently arisen as a response to a contact stimulus of a disagreeable kind, which would be more irritating in a living and moving nestling than in a dead one. It is transitory, beginning to rise on the first to third days, and to wane in the tenth to the fourteenth.

5. The American black-billed cuckoo is born with rudimentary down, which never unfolds. It has strong grasping reflexes, and is remarkably enduring. It can hold by one leg or toe, for a surprising length of time, and draw itself up to the perch with one or both feet, at birth or shortly after, powers which no other birds in this part of the world are known to display, and which must be regarded as preparatory to the climbing stage soon to follow.

6. On the sixth day the complete quill stage is reached, when the bird bristles with feather-tubes, which bear at their apices the white hair-like tubes of the down. The preening instinct has then asserted itself, and the horny cases of the feather-tubes, giving way to their bases, are rapidly combed off by the bill over the greater part of the body. The wing- and tail-quills, as well as some of the contour-feathers are released in the usual way, centripetally from their tips.

7. Fear is attuned to the climbing stage, and not to that of flight as in all the common altricial birds, and matures with comparative suddenness on the sixth day, or shortly before the bird is ready to climb.

8. Parental instincts are as strong in the American cuckoos as in thrushes or in passerine birds generally, and there is no more indication of a retrogression to parasitism in the former than in the latter.

9. The nests of these cuckoos, though slight, are well adapted to their purposes, and often long outlast their use.

10. When disturbed in its nest-activities, the black-bill has been known to transfer its eggs to a new nest of its own, an action which strongly suggests the practice of the European cuckoo of carrying its laid egg in bill to the nest of a nurse.

11. The American species occasionally 'exchange' eggs, or lay in other birds' nests, and when so doing the black-bill has been known to struggle for possession of the stolen nest. Since similar actions have been repeatedly observed in one or another degree, in numerous species, in which no suspicion of parasitism exists, and in all parts of the world, they must be ascribed, in addition to the reasons given above, not to "stupidity or inadvertance," or to "a tendency towards parasitism," but to temporary irregularities in the rhythms of the reproductive cycle.

PLATE I

EXPLANATION OF FIGURES

1 Young cuckoo on day of birth, and two eggs, which were hatched two and four days later, respectively. From nest no. 2 (fig. 9), August 1, 5 p. m. One-half natural size.

2 Young cuckoo, shortly after birth, raising itself to support. Bird no. 3. nest no. 3 (table 6); born 12.45 p. m., July 20; photographed at 4. 30 p. m. Two-thirds natural size.

3 The same bird hanging from second and third toes of zygodactyle foot. Photographed on same date, at same time, and same relative size.

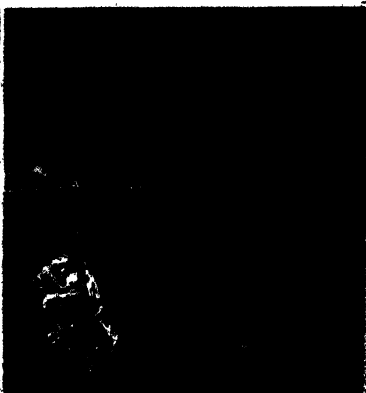
4 Cuckoo, seven days old, sitting on nest, with feathers partially erect. Bird no. 3, nest no. 1 (table 6), July 29. Would probably have left the nest earlier but for slight injury.



1



2



3



4

PLATE 2

EXPLANATION OF FIGURES

5 Cuckoo giving feeding reaction shortly after birth, before association with nest and parent has been formed, showing peculiar pattern of white pads on palate and tongue. Same bird shown in figs. 2 and 3, and photographed on same date, at same time, and in nearly the same relative size. Rudimentary down feather-tubes of back erect.

6 Young cuckoo in typical "quill stage," showing toes clenched, and "flagellate" feather-tubes of contour feathers, before any have been released by the combing process. Bird no. 1, nest no. 1, July 25, 4.15 p. m.; combed off feather-tubes, and left nest the night or morning following before 9.10 a. m. One-half natural size.

7 Contents of nest no. 1, July 23, 11 a. m. (table 6), showing birds nos. 1, 2, and 3, and egg (no. 4). Only the smallest bird, which was but a few hours old, would give the feeding reaction, when all were removed from the nest. This bird reacted promptly at a given stimulus for the space of twenty minutes. One half life-size.

8 Cuckoo in typical climbing stage, shortly after leaving nest. Bird no. 2, nest no. 2, Aug. 10, 9.30 a. m. This bird was in the quill stage (fig. 6) at 6 p. m. the day before. Feather-tubes on head and neck not yet fully released; wing-quills free for one half their length; one tail-quill only broken at tip. Bird no. 1 (11 days old), seen at the same time, but though unable to fly, it could not be captured

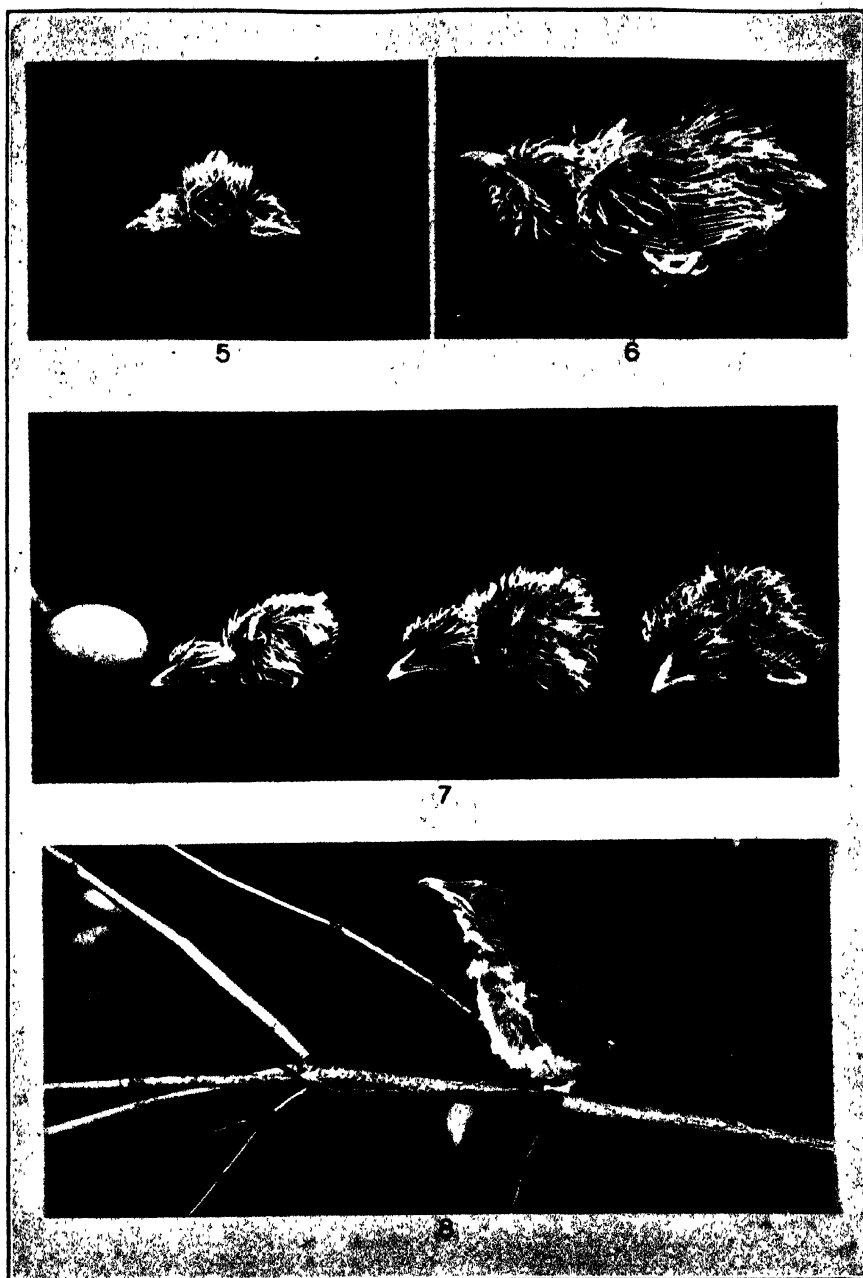


PLATE 3

EXPLANATION OF FIGURES

9 Cuckoo nest no. 2 in sapling white pine, 25 inches from ground, showing birds nos. 1 and 2, three and one days old respectively, and egg (no. 3), which was hatched on following day. Aug. 4, 5. p. m.

10 Young cuckoo in climbing stage, showing attitude, when in falling it will catch a twig and hang by one foot, before recovering itself. About 11 days old.

11 Young cuckoos in nest showing fear, spreading, bristling and giving the alarm call. Birds nos. 2 and 3, nest no. 3, July 27, 12.15 p. m. After this photograph was made, both jumped out of the nest, bristled when touched, and refused to remain, when returned.



PLATE 4

4 EXPLANATION OF FIGURES

12 Female cuckoo cleaning nest, sac in bill. Nest no. 1. $\frac{1}{6}$ natural size.

13 Female cuckoo feeding hairy caterpillar to young; "mouth-reaction." Insect held in mouth simply, from one-half minute to five minutes, before swallowing reflex ensues. About $\frac{1}{4}$ natural size.

14 Female feeding young. First movement in "throat-reaction". Here a grasshopper is inserted in the mouth. Nest no. 3, July 26, a. m., after bird no. 1 has climbed out. About $\frac{1}{4}$ natural size.

15 Female serving gray moth, "throat-reaction," final position before swallowing reflex comes. Nest no. 3, July 25, 5.51 p. m. Remaining egg which was addled, is now completely neglected, and was found on the ground the day following

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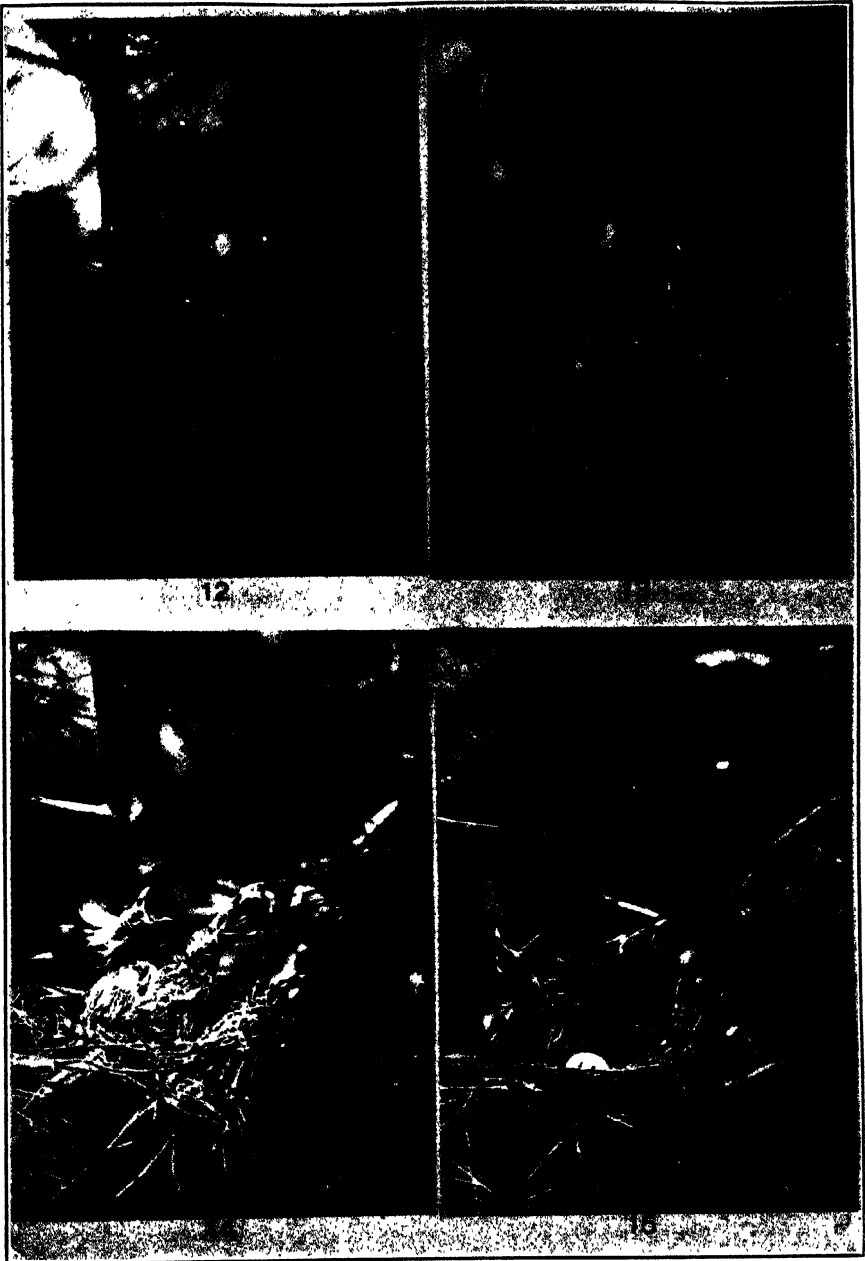


PLATE V

EXPLANATION OF FIGURES

16 Female, having fed young, in attitude of inspection, nest no. 3, July 26. About $\frac{1}{4}$ natural size.

17 Female in brooding and partial shielding attitude. July 25. Figs. 6 and 17 with full lens, Zeiss, ser. ii, a, in full sun, at distance of 29 inches.

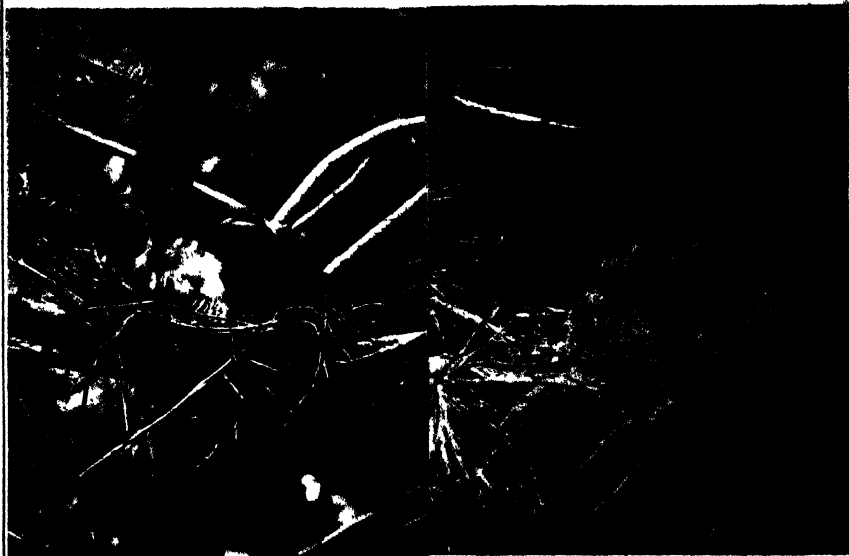
18 Brooding attitude, throat swollen, after rain, and wings partly spread, July 24, 12.53 p. m.: 1 sec. in dull light. About $\frac{1}{4}$ full size.

19 Bird to right combing feather-tubes (bird no. 1, nest no. 3) 9.56 a. m., July 26. A minute later it jumped out of the nest, caught on a branch and pulled itself up; later dropped to the ground and made off. About $\frac{1}{4}$ natural size.



16

17



18

19

PLATE 6

EXPLANATION OF FIGURE

20 Female brooding in dull light, when rain was beginning to fall. Nest no. 3, July 24, 10.40 a. m. This bird came to the nest quietly, and without food, gathered her three young under her, to the neglect of the egg, which is seen brushed to one side, and brooded 15 minutes until called off by the male. 1 sec. with full lens, in very dull light. $\frac{1}{3}$ natural size.

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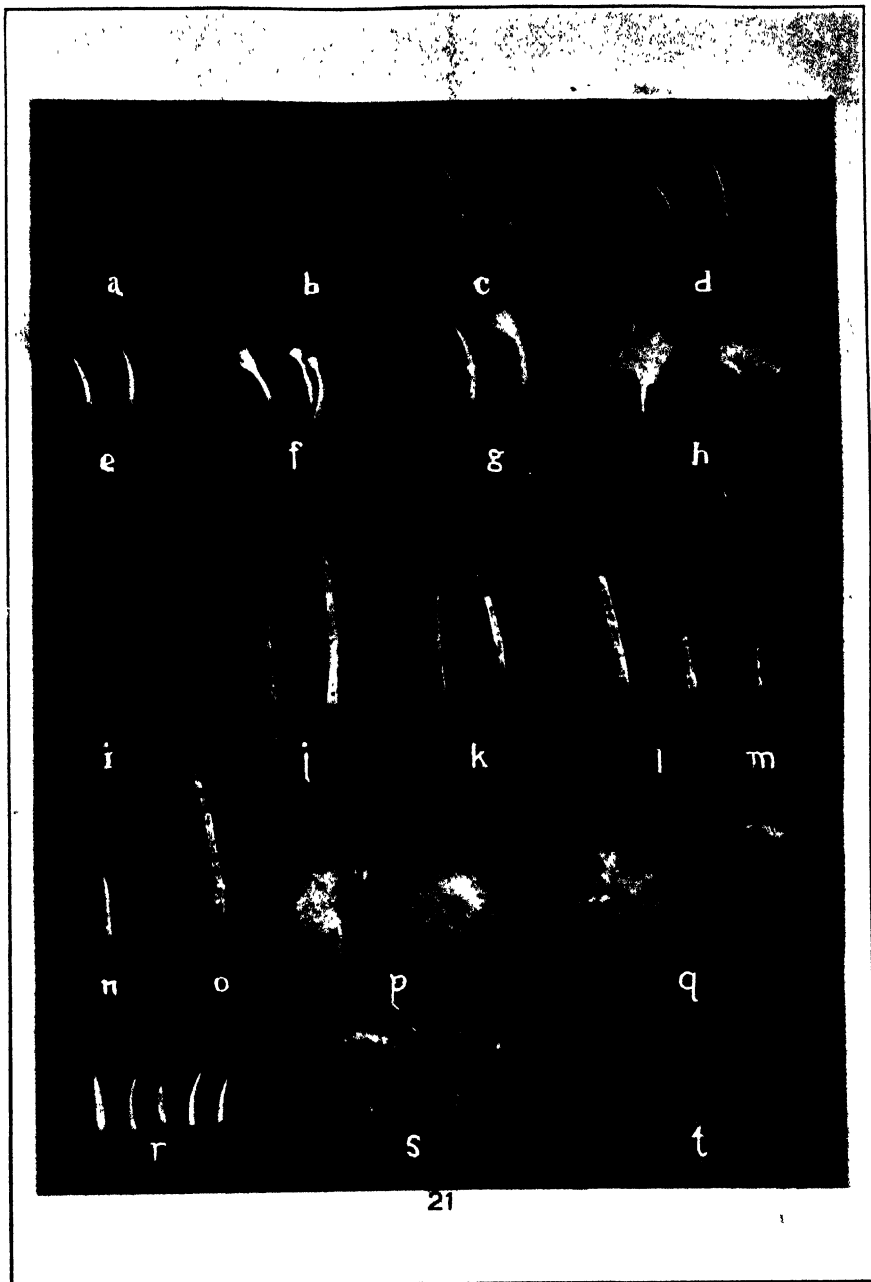
PLATE 7

EXPLANATION OF FIGURES

21 Illustrating the rudimentary down feathers at birth, and the growth of the flight and contour-feathers from birth to climbing stage.

a, Down feather-tubes from dorsal tracts of cuckoo at birth; bird No. 4, nest No. 3, July 25. **b**, Down tubes from femoral tracts from bird a few hours old. Bird 1, (shown in fig. 1), nest 3. Aug. 1. **c**, The same, dorsal tracts. Bird 1, nest 3, July 25. **d**, The same from femoral tracts, showing long flagellum of rudimentary down; bird 1, nest 2; beginning of 6th day, in quill stage. Aug. 6th. **e**, Feather-tubes from abdominal tracts from same. **f**, Feather-tubes from abdominal tracts; bird 4 (quill stage); nest 1, seven days old. Aug. 1. **g**, The same: bird 2, nest 1; 6th to 7th day, July 27. **h**, The same, showing feather released by removal of horny sheath entire; same bird and date. **i**, Two primaries at birth, showing down-tubes; bird 4, nest 1, July 25. **j**, Primaries from cuckoo in complete quill stage; the smaller from bird 1, nest 2; 6th day, Aug. 6; the larger from older bird. **k**, The same from bird in climbing stages; No. 4, nest 1; 7th day. Aug. 1. **l**, The same; bird 2, nest 1; 7 days old. July 27. **m**, Primary from bird in climbing stage, showing feather released for two-thirds of its length; bird 2, nest 2; 8th day, climbing stage. Aug. 10:10 a. m. **n**, Tube from femoral tract; bird 2, nest 1; 7th day. July 27. **o**, Tail-quill; bird 2, nest 2; eight days old, in climbing stage. Aug. 10. **p**, and **q**, Feathers from femoral tracts; bird 2, nest 2; 7 days old. Aug. 9. **r**, Cast off horny sheaths, from litter of nest, bearing down-tubes at tips. **s**, Wing-coverts; bird 2, nest 2; 7 days old; 10 a. m., Aug. 10. **t**, Feathers from femoral tracts; the first to appear; bird 2, nest 2; 6 days old. Aug. 9. All in full size, and as plucked from bird

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CONJUGATION IN THE CRAYFISH, CAMBARUS AFFINIS

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EIGHT FIGURES

The devotion of the individual to the welfare of the race is especially patent in such complex animals as the Arthropods, in which many organs and actions relate directly to the processes of reproduction.

In many crustacea the most complex of all the interactions of the individual with the environment are the interactions with individuals of the opposite sex. A study of the conjugation phenomena of such crustacea should enable us to estimate the highest capabilities of these animals and to place them more accurately in the scale of being, than by study of organs and activities concerned merely with self-preservation. A comparative study of conjugation might aid in the understanding of the relationships of groups of crustacea.

Since the meeting of the sperm and egg will not take place, under usual conditions, without the preceding phases of conjugation, these become as necessary for the race as the fertilization of the egg.

The present paper is a description of the processes of conjugation in a common crayfish, *Cambarus affinis*, in which the sexes differ in many organs and actions.

The female not only lacks the male organs and instincts but possesses ovary and oviducts, a peculiar sperm receptacle in the shell, special glands used in connection with the care of the eggs, characteristic proportions of various parts of the body and character of first abdominal limbs. The female also possesses special

reactions and instincts used both in the processes of conjugation as well as in laying and caring for the eggs.

The male, while lacking the female organs and instincts, has testis and deferent ducts as well as three pairs of external organs directly concerned in the transfer of the sperm to the receptacle of the female. The male shows characteristic proportions of various parts of the body and has a pair of special clasping-hooks. The male also possesses a series of complex abilities that are shown in the processes of conjugation.

The phenomena of conjugation are, in brief outline, the following: The aggressive male seizes the comparatively inert female and mounting upon her ventral surface becomes firmly attached both by the large chelae and by special clasping-hooks on the bases of the legs. The sperm that issues from the deferent ducts through soft protrusions which we shall call the papillae, is conducted by the combined action of the first and second limbs of the abdomen into the special receptacle in the shell of the female,

The male has three sets of organs concerned in the transfer of sperm, the papillae and the two pairs of specialized limbs. These limbs we shall call the stylets, as they are firm, calcified, tapering organs that are thrust with force into the narrow slit of the calcified receptacle in the shell of the female. By means of these stylets the sperm is transferred some half-inch or so through the water from one animal to the other without being exposed to contact with the water, which would, it is believed, destroy the sperm.

During most all the time of conjugation the male is firmly locked to the female in the attitude shown in fig. 1, which is from a photograph of living crayfish under water.¹ The female is supine and relaxed except for the tightly rolled abdomen that is embraced by the tensely flexed abdomen of the male. The male is poised over the female and balanced right and left by the tip of but few legs, while the other legs hold the female, the great

¹ An excellent photograph of a different phase of conjugation in this same species was made from live crayfish under water by R. W. Shufelt and published by him in "Shooting and Fishing," 1898, and in "Natur und Haus," 1903, and by J. Arthur Harris in Sc. Bull. Univ. Kansas, 1903.



Fig. 1 Photograph in water of living male and female in late phase of conjugation.

claws grasping all the claw legs of the female. The apparent absence of the fifth leg of the male is due to its being turned abruptly across between the male and female. Here it supports the styleys, that are seen dimly, pointing forwards and downwards from the abdomen of the male to the thorax of the female.

Once the male is firmly fastened to the female the pair may be lifted out of the water without the male necessarily letting go or even showing any sign of being stimulated by the change in conditions. It is possible to separate the chelæ of the male from the female and to bind them shut without stimulating the male to let go with the other limbs. External environment seems for the time to be concentrated in the crayfish of the opposite sex, as far as evident responses indicate action of environment.

Before considering this process of conjugation in more detail, some consideration may be given the question as to how the male "recognizes" the female.

In *Cambarus affinis*, as studied in the laboratory, the males will unite with the females during all the fall, winter and spring months, and if a male be kept in a small vessel till accustomed to it he will generally conjugate with a female introduced into the same vessel, usually very soon, but sometimes not for twenty-four hours, so that it is not always the advent of a new crayfish that stimulates the male to action. Observation of males and females under these conditions gave the impression that the male has only a vague stimulus from any crayfish at a distance, without any recognition of the sex at all. But once the male had seized another crayfish the result depended upon the sex of the crayfish seized; so that, in a sense, the male might be said to recognize the difference between a male and a female after he had seized them. That is, the male seemed to act differently to males and females only after they had been seized. And even then there was no evidence of any recognition of sex, except in the sense that the mode of reaction of the female made possible the carrying out of a chain of reflexes on the part of the male, which could not be the case if a male were seized.

Incidentally to other observations, Dearborn observed that a specially vigorous male, when blindfolded by a tin helmet,

seized another male, which at once escaped. As he did not see male seize male on other occasions, he came to the conclusion that crayfish use their eyes as one mode of recognizing the sex of their fellows, though he also surmised the sense of smell might play some part in the sex recognition.

While carefully devised experiments would be necessary to disprove the use of the sense of sight and of smell in sex recognition in these crayfishes, there is as yet no evidence that the crayfish recognizes the sex of another with which he is not in actual contact. On the other hand the behavior of the sexes once they are seized is so different that this alone would account for the carrying out of the processes of conjugation between any members of the opposite sexes.

When the males are approached they brandish their claws and if there are two males one withdraws, after more or less deliberate holding and shoving of one another's claws. When a female is approached she generally retreats, but may show fight and more or less successfully keep off the male. In confined spaces, however, the female is almost always overcome by the male even though he be much smaller or lacking in one claw. A few females amidst many hundred males will always be found out and clasped by males; but when very many males were kept together only one case was seen in which a male had seized and held, as if in conjugation, a small male, but this one was dead. That this selection of females by the males may be merely a matter of adjustment of males to the different amount and character of resistance offered by the females and males when seized is, perhaps, supported by the fact that a difference in muscular tonus is rather distinctive of the sexes. In fact it was found possible to sort a lot of crayfishes into two sets, one male, one female, with one's eyes shut, merely by their differences in muscular contraction when taken hold of, the males have a much more pronounced habit of violently contracting their limbs and trunk muscles, so as to pass into a rigid state that may last a long time when the animal is out of the water. The females are, as a rule, notably more relaxed.

When the male actually seizes a female or a male he seems to judge of the amount or the character of the resistance by pushing

his claws back and forth. That the male can thus be misled by the lack of male resistance and continue in the process of conjugation was shown by the above case of a male that was conjugating with a dead, inert male. The following experiment also shows that the male may be deceived, or at least be led to conjugate with a male, even if alive, provided the male acts passively, and does not resist like a male.

A male was given another male that had been operated on twenty-four hours before so that it could not walk but only move its legs, vaguely, as the result of destruction of the brain. This inactive male was at once seized, turned, mounted and treated just as if it were a female for more than hour and a half, during which the active male succeeded in carrying out the usual crossing of the fifth leg, though this was rendered difficult by the fact that the leg on the right was lacking and one on the left lacked two segments. Though the abdomen was contracted and thrusts of the stylets executed, there was no approach to the region where the sperm pocket would be in a female, owing to the fact that the hooks of the male did not engage in the joints of the assumed female. That this was due to some fault of the active male was proved by removing him and manipulating another male upon the paralyzed male, when the hooks were made to engage as if the paralyzed one was female. There is no special organ of the female that receives the hooks of the male, but the same joints in the legs of both sexes may be so used.

The above male after being separated from the paralyzed male was given a female with brain destroyed twenty-four hours and dead for some hours. The male paid no attention to the dead female till it was shoved towards him, when he instantly seized it, turned it over, mounted upon it, grasped its claws, crossed the left fifth leg, contracted the abdomen, and made thrusts of the stylets, all within one minute. But the female's left claw was not held in the male's right and, as if very purposely, he shoved the female's claw up with his second chelate leg then held it with the others in his right. The hooks, also, were not fixed, and five or six efforts were made before one of the hooks was engaged in the joint of the female's second leg. After two hours and a-half the male

was hooked so as to lie diagonally, that is, on the right his hook was in the female's third leg and on the left in the second leg. Half an hour later, however, the male had hooked straight and so continued. Thus at least all the early and possibly all the later stages of conjugation may be carried on with a dead female, which emphasises the passive nature of the female's behavior in normal union.

Again when the males were bound with chelae closed and limbs in the posture assumed by a female in conjugation, other males with bound chelae were excited by contact and, without the usual initiatory use of the chelae, even mounted upon the supine, bound males and endeavored to carry on conjugation.

It is thus possible that from the crayfish standpoint the only difference between the sexes is a difference in behavior and not a difference in form, and moreover a difference received by muscle and touch sense and not in effect upon any of the other sense organs.²

The crayfish is thus much like the amphipods studied by Holmes who found, "The different reactions of the two sexes to contact with other individuals is the factor which effects the union of the males with the females."³

Coming to a more detailed description of the processes of conjugation, we shall describe first the behavior of the female and then that of the male and divide his activities into the following groups: (1) Seizing; (2) Turning; (3) Mounting; (4) Claw clasp-ing; (5) Erection and locking of the stylets; (6) Crossing of fifth

² The observations of Chidester upon the crayfish, *Cambarus bartonii*, confirm this point of view, for he observed that these males repeatedly grasped males and even, despite their struggles, turned them and attempted conjugation. The females when seized firmly ceased to struggle and lay passive. He inferred that the males did not recognize the females.

³ After this paper was ready for press appeared the account of the experiments of A. S. Pearse, in the *American Naturalist*, December 1909, in which the conclusion is independently reached that the crayfish has "little or no power of sex discrimination." His observations were made chiefly upon *Cambarus virilis*, and make it probable that all crayfish lack means of acting differently toward males and females till they are in contact with them and are influenced by their sexually different responses, in the fields of touch and pressure.

leg; (7) Hooking; (8) Advance; (9) Recession; (10) Palpation; (11) Contraction of abdomen and claws; (12) Entrance; (13) Thrusts of stylets; (14) Discharge of sperm; (15) Formation of plug; (16) Withdrawal; (17) Liberation of female. Some of these phases are very brief, some less essential, and pauses of longer or shorter duration come into the series of active states.

The part of the female during all these phases of conjugation, which may consume as many as nine hours, is chiefly a passive one, at least after the initial stages.

When the female is seized she generally struggles as if to escape and also defends herself with her claws more or less vigorously. The actual seizure by the male may well supply a strong stimulus to the female, since the male's chelae frequently close firmly upon the limbs of the female on one side while at the same time holding the rostrum, eyes and bases of the antennae. At this stage the female frequently makes violent leaping movements, backward, which however, may only facilitate the turning of the female over, since the female in these leaps becomes suspended in the water, while the male remains supported upon his legs and has the better leverage.

Once the female is turned over by the male she remains through the following states so passive that she appears dead and the above male that tried to conjugate with a dead female probably missed little reaction from the female. This passive state seems to be like the hypnotic condition which Dearborn says results from the holding of crayfish in any constrained position. In this state there is not a relaxation of all the muscles but a strong flexure of the abdomen, which remains coiled. This coiling of the abdomen is lacking in a dead crayfish and it may be of some aid in the process of conjugation, since the telson of the male is pushed against the coiled abdomen in a way that seems to aid in the leverage that makes possible the thrusting of the stylets, as will be described later.

A striking difference between the male and the female during conjugation is the fact that while the male carries on violent vibratory or fanning movements of the exopodites about the mouth (which may be in part a sign of excitement), the female remains

without these movements and probably has but a small respiratory exchange in comparison with the more active male during the conjugation.

But while the female is so inert the nervous system may be receiving stimuli of some sort. The two minute and apparently useless limbs of the first somite of the abdomen are seen to reach up in the water to the stylets of the male, and in one case to touch the endopodites that had sperm upon them. Possibly some general sensations are received through these palp-like limbs. Possibly study of these organs would show that they are of use as sense organs, though of no great importance. Herrick has suggested that in the female lobster these limbs have been reduced to prevent eggs being attached to them, as that would interfere with the closure of the abdomen over the other eggs; but even granting this, the limbs may have a sensory value, both in conjugation and in egg-laying, though they are not necessary, as I have proved by removing them. After conjugation these little organs reach to the sperm plug, but it seems improbable that the female is aware of the success of conjugation.

During conjugation there are sometimes twitchings of the muscles of the abdomen and when the stylets happen to be thrust against the soft membrane posterior to the annulus there are twitchings of the body of the female that indicate that the apparent hypnotic state is not one of paralysis of all the body. Again since the annulus is pushed dorsally by the stylets, which enter it so firmly that when the male is pulled away the annulus is drawn out as far as the cuticle will allow, it may be that the female has some sense of the change of pressure in that region.

Turning to the activities of the male, they may, as above stated, be resolved into many different phases, the first of which is the seizing of the female. Most of the males in the mating season seem ready to seize any other crayfish, and if they seize females the rest of the conjugation generally follows. The female is grasped by first one and then both of the chelae, though a mutilated male with only one chela can accomplish conjugation. One chela often seizes the head of the female, but here is much variety in the modes

of seizure. Once the chela has taken hold it does not often relax, but the second is added and henceforth the hold is maintained.

Immediately, or sometimes after an interval, the male that has seized a female enters upon a struggle, a sort of wrestling, that leads to the second act, the turning of the female. Viewing this process, it is hard to escape the impression that the male has a purpose in view to which instinct leads with even what looks like intelligence sometimes assisting.

At times one must admire the solution of the problem of turning over another body braced upon ten legs and actively resistant. But again the clumsy efforts of the female seem to bring about a happy chance position that the male utilizes.

Sooner or later the female is upside down and still held by both chelae of the male. The second phase of the activity, the mounting, now follows: It is not known how the male is aware of the inverted position of the female, but the complex actions that follow lead one to believe that the sense of touch and muscle sense give the male a means of quite accurate response to the form and position of the female. The male mounts upon the female so that the ventral surfaces of the two are near together. The male then brings the two into a position in which the median planes of both coincide and, their heads being in the same direction the right of the male is over the left of the female and vice versa, fig. 1.

After this mounting comes the difficult phase of claw-clasping, which seems to satisfy a strong instinct. The object attained is that most all the legs of the female are firmly held in the two chelae of the male. The old hold of the chelae is gradually changed, without letting the female at any time free from the grasp of one chela. Generally in a very few minutes all the left clawed legs of the female are held by the right chela of the male and all the right clawed legs by the left chela of the male.

This feat is facilitated by the habit of the female, when seized, of throwing all the legs forward and upward alongside of the head, so that they are much more readily taken hold of in a bunch than could otherwise be the case. Yet the male that has hold of but few legs generally tries till all are finally grasped, exhibiting what seems a strong desire to hold them all.

The greater size and especially length of the chelae of old males seems directly connected with this function and it is noteworthy that the chelae so nicely encompass all the ends of the claws and walking legs of the female.

However, it is not necessary for the completion of conjugation that all the claws be held. Some may escape the normal male and a male with but one chela can hold the legs of but one side. One male lacking claws on both first and second left legs held in conjugation a female lacking the same claws on the right, but with the one chela of the right the male held all the claws that remained on the left of the female.

Experiments would be necessary to determine the true nature of this habit of claws-clasping. When some female claws are left free they have been sometimes seen to pinch parts of the male.

Mounted thus upon the female, the male is held in position not only by the two chelae but by the contractions of other legs that are wrapped, as far as their rigidity permits, about the convex thorax of the female. There remain but few legs that stand out right and left from the male and prop the body from falling over, fig. 1. As the back of the female is rounded the conjugating crayfish tend to roll over onto the side, but this is resisted by the male legs that act as props. But while the conjugation is commonly carried on with the male in the normal position of locomotion and the female in the forced, inverted position that crayfish assume only under compulsion (except at the period of egg-laying), it frequently happens that the pair lie upon their sides. When the water is so shallow that it will not cover the male as above mounted, the pair may lie upon the side, and thus conjugation was carried out in water too shallow to cover even one animal.

But before the firmest clasping of the female the male must erect the stylets, cross the fifth leg and attach the hooks. These three acts take place as follows:

When the male has seized all the claw-legs of the female in his two chelae he moves back and up away from the female, still holding the claws, and then raises the organs that are to transfer the sperm. These are the first and second appendages of the

abdomen. Crayfishes have the habit of swinging back and forth the small appendages found under the abdomen; in the female this serves to aërate and clean the eggs and embryos when they are attached to these appendages, but at other times, and in the male at all times, it is not obvious what use the swinging motions of the "swimmerets" may be. However, when the peculiar male appendages are to be used the entire series is set swinging and the first and second partake of this motion enough to be raised from their usual position to the one of use in sperm transfer. These two appendages at rest are carried by the male forward, horizontally, in the deep groove under the thorax where they are concealed and protected. However, at this stage of conjugation they are lifted up by their muscles, or really swung downward and backwards as far as their stiff basal joints will allow, only about 45 degrees from the horizontal. A slight muscular movement serves to place the second appendage against the first so that it locks into it. The normal position of these organs is horizontal and they tend to return to it, but during the following hours of conjugation they are held up by a remarkable device that relieves the weak muscle that elevates the stylet from the task of holding the stylet erect. The male with difficulty and care crosses either the left or the right, or sometimes first one and then the other of the last or fifth legs, under his thorax, in spite of the near presence of the body of the female that makes it awkward to swing the limb across; and thrusts it over between himself and the female till it lies as flat as possible under his thorax with the terminal joints protruding beyond the opposite side of the body. Henceforth in conjugation the male seems, at first sight, to lack the fifth leg on one side (fig. 1), while careful observation shows its tip on the other side. Either first or later trial has placed this leg across anterior to the erected stylets, and in this position this leg holds the stylets mechanically firm and erected at 45 degrees till the end of conjugation, when the leg is moved back and the stylets allowed to fall into their usual horizontal position of rest. Often, however, during the early stages of conjugation the male will try first one and then the other of the fifth legs in the attempt to carry on the chain of reflexes to the consummation of the fitting of the stylet into the receptacle.

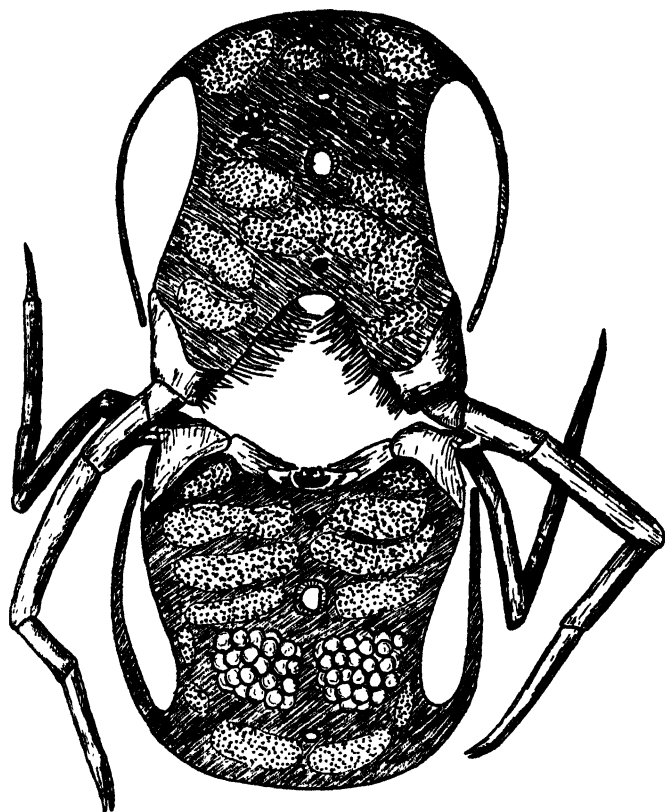


Fig. 2 Diagrammatic cross section of male and female in conjugation to show mode of attachment of hooks.

After the above preparation the male settles down against the female and tries to fasten the hooks to the bases of the female legs. Holding the left legs of the female in his right claw and her right legs in his left claw the male tries to make fast the hooks that are on the third joints of the third legs to the grooves at joint of first and second segments of the third legs of the female. These grooves are accentuated by the fact that the female legs are bent up dorsally. In accomplishing this the male no longer stands up high above the female but brings his ventral surface as near as possible to the thorax of the female. If the two animals are at

all nearly the same diameter it is finally possible for the male to catch the hooks into the joints of the female as indicated in the diagram of a section of the two conjugating animals (fig. 2). Really it is only the base of the legs that are in contact, since the ventral side of the males is hollowed out, as represented in the diagram by the groove lined with setae. And in this groove lie the two pairs of appendages of the male that are soon to be used.

The hooks hold so firmly that lifting the male out of the water lifts the female also, and indeed it is impossible to separate the two without unlocking the hooks.

Usually all the stages of preparation so far considered, including the hooking, require but a few minutes, but with great individual differences.

With the male and the female firmly locked together and the stylets held at an angle of about 45 degrees, the transfer of sperm can take place after the muscular efforts of the male have forced the tip of the first stylet into the firm orifice of the annulus of the female. To accomplish this the two animals must be quite accurately adjusted and this requires some trial. There is more or less advance and recession of the body of the male along the female till the tips of the stylets find the mouth of the sperm receptacle. In these trials there is sometimes seen a rapid play of the setose tips of the third maxillipeds, quick sidewise and lengthwise motions over the bases of the legs of the female. It seems that some information may be acquired by the male by this palpation.

To obtain actual entrance of the tip of a stylet into the receptacle, force is finally exerted by violent contractions of the muscles of the male. The posterior part of the abdomen of the male grasps the coiled-up end of the abdomen of the female as a hand about a ball (fig. 7) and tends to hold these ends of the animals together. And at the front end the chelae hold the claws of the female. Between these two points the hooks (fig. 2) bind the two animals immovably. When the abdomen contracts the long chelate legs also contract without losing their hold and they tend to straighten out. This elongation of the chelate legs tends to throw the head of the male up away from the female. As the

hooks do not yield and as the thorax and abdomen are held as one piece by the contraction of the powerful muscles that connect them, the result of the unbending of the chelate legs is to depress the base of the abdomen, to force it nearer to the female. This is resisted by the rigid stylets, but if their tips are at the mouth of the receptacle they must be driven into it.

This see-saw action of the whole animal that brings all the muscles into play to force the stylet to enter the annulus will be considered again after describing the anatomy of the hooks.

To bring the organs to the position in which the actual transfer of sperm can take place thus requires the exertion of much muscular force in carrying out the instincts of the male.

As will appear in another paper the mechanism of sperm transfer along the stylets is not completely understood. Besides the contractions of the sperm duct there are movements of advance and recession of the second stylet upon the first that have some significance and there is still another action, the movements of the abdomen that cause several successive short thrusts, or tamping movements of the stylets.

The last four phases—discharge of sperm, formation of plug withdrawal, liberation of female—were but little studied. Many hours are consumed before the sperm receptacle is quite filled, and the actual packing of it is removed from observation. It does not appear that the sperm leaves the male till the stylet has found entrance into the annulus, and it is rare that any of the sperm escapes into the water.

Normally all that is discharged goes into the receptacle, where sections show it arranged as if it had flowed in as a liquid mass. However, only the innermost part of the receptacle contains pure sperm and the outer parts are full of a secretion that is finally added in excess so that it protrudes from the mouth of the receptacle, as a sperm plug, that is evidence of conjugation having taken place.

After the receptacle is filled the male reverses some of the preliminary phases by first raising the bases of the legs so that the hooks are disengaged from the grooves of the female's legs, then rising up away from the female, then crossing the fifth leg back

into its usual position, then letting the stylets recede to their usual horizontal position and finally letting go of the claws of the female.

As soon as the claws of the female are released she returns to the customary position with the ventral side down and keeps this with great persistence except in the processes of egg-laying and turning, elsewhere described by me.

Though the stylets are the essential ducts for the transfer of sperm, it was found that when they had been cut off entirely the male would still carry on the preceding stages of conjugation and even contract the abdomen as if thrusting forward the stylets that were not there. While the series of events in conjugation may be thus carried on for a long time, though the end events will be impossible, it is also true that the series may go on for a while when the first part is lacking, or at least but dimly represented. Below it will be shown that the series may go on when a middle factor, the fastening of the hooks is omitted by the removal of the hooks, which prevents the success of the final acts, though they are attempted.

The experiments that show that the perfect expression of the first of the series is not necessary for the carrying on of subsequent parts were as follows: Ten males and females with chelae tied shut by elastic bands were kept separate for several days in water at 20 degrees C., though in December. When the females were put in with the males, each in his accustomed dish, the males acted individually, but most of them tried to seize and turn the females. The males generally remained quite inert till touched by the female crawling about in the strange dish, but a few males rushed at the females before being touched. That they tried to turn the females was seen in the pressure exerted by their claws and turning of their own bodies. The females and males fenced together, or the former sprang backward, as if their claws were not bound, except that there was not the usual open claw and pinching. Without this seizure the males did not succeed in turning the female, though one nearly did so and proceeded to mount upon and embrace the female, though the claw grasping stage was lacking. When the females were bound with the legs

in the conjugating attitude the males were little excited by them till moved about by a pair of forceps, but even then were less excited than by the normal moving of the female. One male mounted upon such a tied female that happened to lie supine and then struggled to carry on conjugation, advancing and receding over the female, grasping with the legs and abdomen and violently palpating with the third maxillipeds. Finally, when, in the pressure of the male's chela along the female's chela, the bands came off, the male used the chelae normally.

In another case, however, the male, though having lost one chela, used the single bound one so effectively that a female with chelae bound but otherwise free was conjugated with rather completely. That is, the female being found upside down was mounted and held. She lapsed into the normal inert state, however. The male mounted and grasped with legs and abdomen and crossed one fifth-leg above the erected stylets, then hooked to the female and made tamping movements with the abdomen. After that the body was elevated in front and force exerted to drive the stylets in. The male shoved the single bound chela against the face of the female and thus made a substitute for the usual grasp.

This attempt at complete conjugation without normal use of chelae lasted an hour and more and the fifth leg was changed to the other side. Eventually the male desisted and though sperm had been seen to issue from one of the first stylets there was no sperm plug in the annulus.

Evidently, however, the male with bound chelae neither is lacking in keen response to the female nor in instinct to carry on as many of the phases of conjugation as possible, though the first acts may be represented only by internal phenomena without complete external expression.

Coming to the anatomical side, we find three pairs of organs in the male absolutely necessary for the transfer of sperm in these complex processes of conjugation, and there are also in this crayfish two spurs or hooks on the legs, the importance of which has not been described. We will here describe only these hooks, reserving an account of the anatomy of the other organs more

directly concerned in sperm transfer for another publication. These hooks are mere hard tubercles, or blunt spines on the shell of the third segment of each third leg, (counting the chelate legs as the first of a series of five legs) fig. 3.

Many other kinds of crayfish have none, others have one on the second as well as on the third leg, while others have one on the fourth as well as the third leg.

The hook is thus not an organ absolutely necessary to crayfish conjugation, though we shall attempt to show that it has become necessary in *C. affinis*.

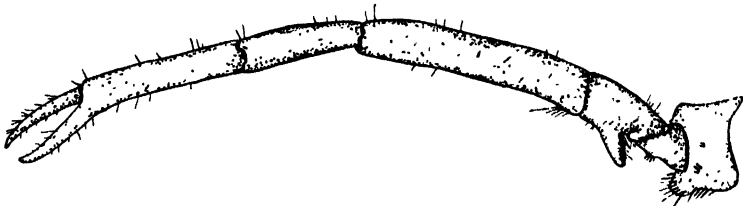


Fig. 3 Posterior face of left third leg of male 64 mm. long.

In the female there is no hook and in the male there is no similar protuberance on the other legs, so that in *C. affinis* the organ is not metamerically repeated, while the other cases referred to show that it may be repeated. Moreover the spine is not a specialization of something found on other legs in that region but is in each case a perfect spine or nothing. To be sure there are similar spines on the big claws and on the body, but not in the region corresponding to the hook.

In *C. affinis* the hook or spur (fig. 3) makes a very conspicuous projection from the proximal part of the third segment of the leg, forming a strong cone with its proximal face flattened, and sparsely set with setae. The whole is white and bony with the tip especially so and as if rolled over as a terminal ridge (fig. 4).

Springing from the underside the spur juts downward and also much toward the body and is so long that in old individuals (fig. 5) it runs far across the line of joint between the second and third segment and tends to become more parallel to the leg.

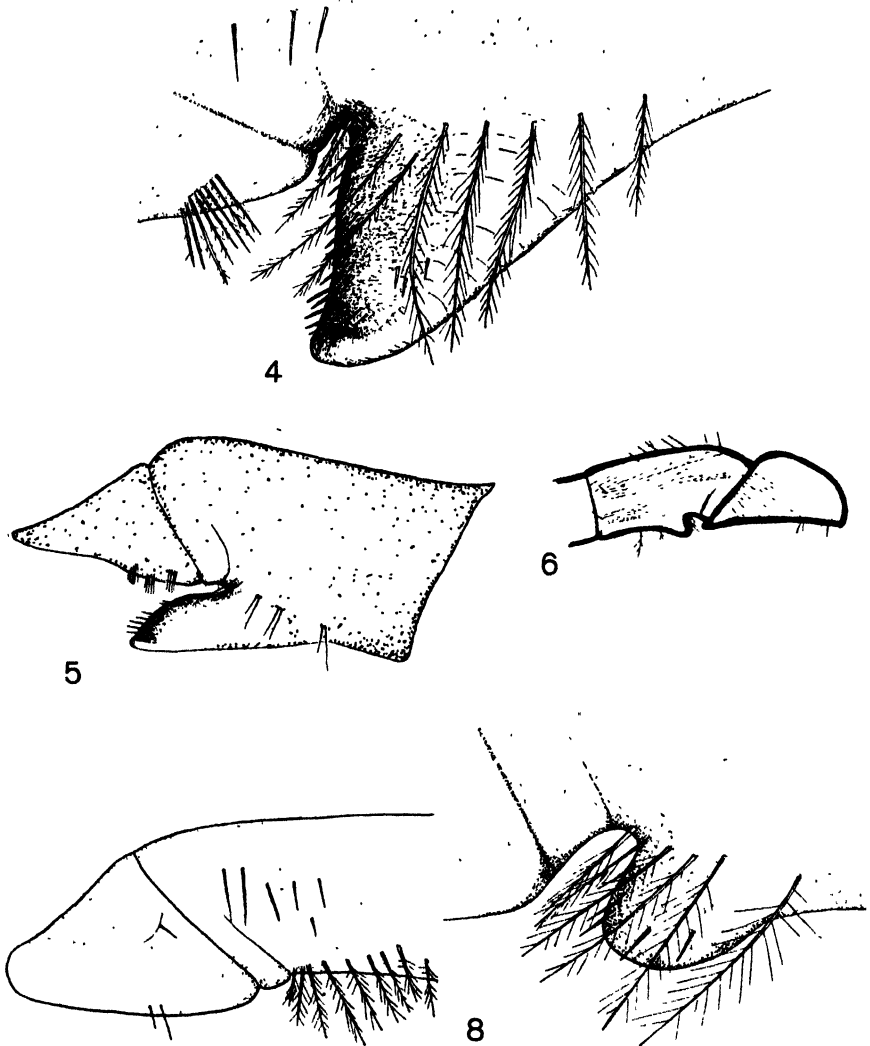


Fig. 4 Anterior face of the hook on the same leg.

Fig. 5 Anterior face of base of left third leg of male 195 mm. long.

Fig. 6 Section of base of third left leg of young male 48 mm. long.

Fig. 7 Anterior face of base of third left leg of male of 22 mm. Oct. 4th, 1904, showing no hooks as yet developed.

Fig. 8 Spur and setae upon base of leg shown in section in fig. 5.

There is thus with age an increase in the size and efficiency of the hook, for the more the tubercle becomes recurved the more is it fit to be a hook that will hold strongly to the female as will be seen presently. In old hooks, fig. 5, the base extends nearly the whole length of the third segment of the leg.

Sections, such as fig. 6, show that the hook is hollow; that is, it is an outgrowth of the shell, lined by epidermis and filled with connective tissue, as are the large tubercles elsewhere on the shell. The hook is thus a special employment of a local outgrowth just as the claw is a special use of the opposition of the terminal segment of the leg to a like tubercle, or outgrowth that opposes the last segment.

In the development of the male *C. affinis*, the hooks come quite late and are perfected after they have been used.

Crayfish of this species reared from eggs laid in the spring and measuring in October 22 mm., had no hooks (fig. 7), but merely the appearance of a false joint parallel to the real joint between segments two and three and a row of plumose setae in the region of the future hook.

But in males of 48 mm. of the same date (fig. 8) there is a large rounded lump with some of the above setae upon it. The section of this specimen (fig. 6) shows the thickness of the shell that continues over the spur and also the arrangement within the second and third segments of the muscles which thus have no connection with the region of the hook.

In the outgrowth of the spur the large setae are left about its base and some small ones are developed over its proximal face. These are well seen in living specimens with shells not too much worn, as in the specimen of 64 mm. shown in fig. 4. In many specimens, as in fig. 5, the setae are broken off more or less. These setae are long enough to suggest that they serve as sense hairs or as tactile setae, which may be of use in enabling the male to adjust the hook to its socket. The short ones along the proximal face may also serve pressure sense.

The above male (fig. 3 and fig. 4), being killed February 1st, had doubtless used its hooks in conjugation, as that has been found to take place in the first fall when the males are but 50–60 mm. long.

The way in which these strong hooks are used needs to be more clearly shown. The bases of the third legs are lifted up and away from one another by the male when mounted upon the female and then brought toward one another till, like spurs, the hooks fit into the joint between the second and third segments of the female's legs. This can be easily done, manually with dead or living crayfish, if only the legs of the female be held up against the sides of the thorax. The joint between the second and third segments of the legs is a soft membrane that can be displaced and the hard hook shoves it in. The face of the hook then comes against the hard rim of the second segment. The rim of the joint shows a small tubercle both on the first and the second segment. When the limb is extended these hinge tubercle are side by side, but when the limb is flexed up against the body the two tubercles together make a stiff, semi-circular rim about the soft membrane. It makes as it were the hollow of an elbow into which the hook enters and out of which it cannot come sidewise because the above hard rim opposes the flattened concave, or proximal, face of the hook. The overhang of the tip of the hook (fig. 4) seems advantageous. The semi-circular pit of the female's leg exists only when the leg is bent. Hence, when we hook two dead specimens together the weight of the female may be held up by the male hooks, mechanically, without muscular effort, as long as the limbs have the general positions shown in fig. 2; but if the leg of the male is raised, or if the leg of the female be straightened out the hook is loosened and the female drops off.

The mechanical nature of this use of the hooks is, poorly, indicated in the section, fig. 2. This shows, however, the inverted female containing ovarian eggs and the male with the coils of efferent ducts in like region of the body, poised upon the female. The bases of the limbs only come into contact, while the ventral surfaces of the bodies of the animals are separated by the space caused by the concavity of the ventral surface of the thorax of the male especially. It is across this space that the stylets will be extended to reach the annulus, which is indicated on the middle of the upturned surface of the female. The hooks on the legs of

the male are so thrust in, horizontally under the rim on the joint of the leg of the female, that any force that tends to push the ventral surfaces of the two animals apart will be resisted by the rim till it should break. If, however, the leg of the male be thrown out away from the side (raised up) the hook will be disengaged.

The female is held much as a piece of ice by tongs that tighten as the weight increases, or as a stone lifted by hooks in holes in its sides with converging chains.

That this use of the hooks is a necessary part of the conjugation in *C. affinis* seems demonstrated by the result of removing the hooks. As they are but protrusions of the connective tissue and epidermis covered with hard shell they can be cut off without serious injury to the animal and the animal retains all its usual eagerness for conjugation.

When normal males are kept with females there is usually a sign that conjugation has taken place in the presence of the sperm plug. But when eight males had one or both hooks removed and, after two to four days, were given females no sperm plugs resulted. The cause of this apparent failure in spite of the fact that many of the males were seen to seize the females was made out by observing several of those with one or with no spurs.

The male crayfish without any hooks seized the female eagerly, turned her, grasped her claws, raised and locked the stylets, crossed the fifth leg and made both the maximum contractions of the entire body and then six or seven quick thrusts of the stylets, just as if hooked to the female. That is, the phases of conjugation went on as usual; the hooking being absent, the next stage was carried on. But a halt came when it was found that the stylets had not entered the annulus. This entrance is a difficult act that usually requires repeated trials. In these crayfish with no hooks the maximum contraction resulted in the elevation of the male a half inch above the female. When the male had settled down close to the female as if to fasten the hooks he then passed to the state of violent contraction. The abdomen held by muscles formed one mass with the thorax, the tail was strongly applied against the coiled up tail of the female, the anterior part of the male abdomen was bent downward so that the stylets were

thrust down and forward, held stiff by the fifth leg, and at the same time the long chelate legs straightened themselves out. This last action, with the others, necessarily pushed the body of the male as one mass up away from the female. Had the body been hooked it would have acted as a lever about the hook as a fulcrum and thus the rising up of the anterior end of the body would have made the posterior end move down; this in turn would have thrust the tips of the stylets forward as well as downward and so have helped in the entrance.

Diagrammatically expressed, the normal use of the hooks is to hold the middle of the male firm so that the body of the male can move on the hook region as a see-saw. When the chelæ shove up the head end of the see-saw the other end goes down. The stylets being held firmly at an angle of 45 degrees tend to push against the ventral surface of the female both downward and forward. The strength of the chelæ is utilized as an aid to the entrance of the tips of the stylets into the sperm-pocket. All the most powerful muscles of the male seem necessary for sperm transfer.

When the hooks were not there and there seemed no way to hold the body of the male firmly to the female at the middle of the length of the see-saw, then the force of the chelæ raised the male away from any possibility of getting the stylets to the annulus.

The absence of the hooks thus led to a separation of the male and female thoracic surfaces, and nevertheless the stylets high in the water above the annulus were then seen to be quickly jerked forward several times, as if to enter the annulus. But the male next acted as if missing the usual response; he endeavored to hold the female tightly with his claws and legs that encompassed her thorax; and after some efforts turned the small antennae directly down and backward under his head as if in search of impressions. The third maxilliped was also used in rapid palpation of the bases of the female legs.

Another male with no hooks after such a first trial with the right leg crossed stood up an inch from the female and substituted the left leg and then went through the maximal contraction-phase

with like failure to bring the stylets to the annulus. The vain efforts continued more than an hour.

A male with only the left hook removed succeeded in attaching the right hook to the joint of the female's leg but, when the strong contraction followed, the hook came out, not being held by its opponent force on the opposite side of the body (see fig. 2), and then the male was thrown off as before. In this male with one hook the animal was seen to change from the right to the left and back again to the right leg after vainly trying to make entrance. This is taken to indicate that the normal male is in the habit of changing legs, as is seen, for the purpose of obtaining perfect entrance of the stylet, whether there is a right or a left annulus presented, and to indicate that but one of the stylets is used at a time.

After the male with one hook had made several quick thrusts of the stylet and encountered no resistance he always settled down close to the female and made exploratory palpations with the maxillipeds over the bases of the female legs. In one case after this examination of the bases of the second and third legs, a few rods of sperm were seen upon the telson of the female, showing that abnormal, premature discharge of sperm had taken place though the stylets had not been introduced.

In these males without hooks failure to gain entrance of the stylets was followed by advance of the body and palpation of the bases of the legs of the female before the next attempt. This raises the question whether in normal conjugation the male may have a complex knowledge of the form of the female, or may judge by sensing the surface of the female whether a change of position will lead to better results. At all events after failure there is the use of sense organs and then renewed trial.

Bearing upon the conjugation of this one species are the recorded facts as to conjugation in other species and the following facts as to attempts of one species to conjugate with another.

It will be shown elsewhere that the stylets in probably all Cambari are alike in use and essential structure despite remarkable differences in general proportions and external appearance.

We have observed conjugation in *C. affinis*, *C. virilis*, *C. clarkii*, *C. immunis*, *C. bartonii* and A. E. Ortman in *C. obscurus*;⁴ and as far as observed the process is like that in *C. affinis*, though the details have not been studied.

When some seven male and only one or two female *C. virilis* were kept with about two hundred *C. affinis*, the individuals of the former species found one another and the female *C. virilis* was conjugating with a male *C. virilis*.

While the males thus select the females of the same species, as far as these observations show anything, it is yet possible for the conjugating reflexes of the male to be brought into action toward a female of the other species. Thus when a male *C. affinis* from which the first and second stylets of the left side had been cut off was about to seize a female *C. affinis* in the corner of a dish, the female was removed and a female *C. virilis* put in its place. The male drew back, stood as if looking at the new female, went to the other end of dish, and in a few minutes returned to face the female and rest his long antennæ upon her thorax for a few minutes, then suddenly seizing her tried to turn her. In a few minutes the male was mounted upon the passive female and holding her chelæ, but not her other legs. The male then made attempts to get more and more of the claws of the female in his claws, at times holding most of them, and meanwhile made quick use of the third maxillipeds to feel over the ventral face of the thorax of the female. The claw of the second leg was also passed over the median part of the thorax of the female. But the male then desisted and the female remained passive, turned first on the side and then to the ventral face, but still held by the right claw of the male fast to her right claw and by the left claw of the male fast to her rostrum. Ten minutes later the male was mounted again and held all the chelate legs of the female in his claws, except one on the left. Half an hour later the male had crossed the left fifth leg anterior to the stylets which remained on the right side, having been cut off from the left side. Yet

⁴ Pearse has studied the conjugation in *C. blandingii*, *C. diogenes*, and *C. virilis*; Am. Nat., Dec. 1909.

a half hour later the right leg was crossed anterior to these remaining right stylets. In this state the right second stylet was seen to swing back and forth by its own muscles through 70 degrees, quickly, before locking to the first. Two hours later the male had separated without leaving any sperm plug, but as this same result always followed when the males were thus mutilated the male had carried out the process of conjugation on the female of another species as far as would have been the case on the same species.

The possibility of crossing is thus good, and its failure would come from some mechanical difficulty rather than from the lack of the instinct to conjugate with any passive crayfish. Of course the fertilization of the egg may be ruled out by some inability of the egg and sperm to combine, but it seemed worth while to fill the annulus naturally or artificially with sperm of another species and await the result the following year.

The sternal plates of many females were cut off and fastened to females of another species, so that these now had their own sperm receptacle replaced by that of another species. But though some of these mutilated females lived to lay eggs, the eggs did not develop and the experiment failed. Attempts to have such transplanted receptacles filled by males also failed.

The male of *C. virilis* will also respond to the females of *C. affinis* as is shown by the following: A female *C. affinis* was put into the dish inhabited by a male *C. virilis*. The male in two hours had seized the female and was holding her chelate legs in normal manner and was trying to get the left fifth leg in advance of the stylets, which was difficult on account of the fact that the great length of the stylets of this species made them strike against the fifth leg, which was held with the elbow, or joint between the third and fourth segment from the tip, pressed against the thorax of the female. But finally the male rose up far enough away from the female to allow of the stylets being placed anterior to the fifth leg. While making these efforts the first and the second stylets were fastened and unlocked several times; when the second stylets were free they were swung back and forth quickly, while the first rested forward horizontally under the thorax. Then the first were raised a short way by their own muscles, but then be-

coming locked with the second both together were raised as far as possible to be at right angles to the body. The right and the left stylets acted together, yet one often moved a little ahead of the other for a short distance only.

These preliminary experiments show merely that conjugation between species may take place to some extent. Whether sperm transfer may be thus accomplished remains for future experiment to show.

This is of import here merely as showing the general nature of the instinct of the male and the similarity in the structure and use of the organs of sperm transfer in the different species of *Cambarus*.

CONCLUSIONS

The most complicated activities of crustaceans are those processes of conjugation that bring the sperm where it may ultimately meet the eggs.

In the crayfish, *Cambarus*, the sperm though injured by water is transferred from male to female while under water. The protection of the sperm involves many habits and organs of male and of female. In the female a peculiar receptacle stores the sperm till special habits and secretions bring it safely in contact with the eggs outside of the body. In the male three pairs of organs are devoted to the protection of the sperm while being transferred to the female.

The processes of conjugation in this crayfish involve the accurate adjustment of the entire body of the male to the body of the female when placed as a mirror image of the male.

The female plays a very inactive part while the male contracts most of the muscles of the entire body and limbs.

In *Cambarus affinis* the pair of hooks on the third legs are necessary for sperm transfer and hence for the continuation of the race. They become fastened so as to form a fulcrum about which the muscles of the claws and body act. In structure they are but elevations of the body-wall like other spines, but in this species they have no homologue on other legs.

They arise in the first year and become more developed after their first use.

The female has no special organ to receive the hooks, but the joint mechanism common to both sexes is used as a socket, which is efficient as long as the males and females keep their special postures in conjugation.

The fifth leg of the male is also used as an auxiliary organ as it furnished mechanical support for the stylets. Its use on the right and the left is guided by remarkable actions of the male.

The length of the claws of old males seems advantageous for carrying on conjugation.

In conjugation there is a series of events advancing in orderly sequence to the transfer of sperm and then, in part, reversing. In this series there may be considerable trial of various organs at various stages and this varies with individuals and cases.

The early stages of conjugation are carried out after the removal of the stylets has made the completion of the series impossible. Later stages are performed after removal of antecedent middle stages. After very imperfect expression of the first stages, later stages may follow as far as is mechanically possible.

The male acts as if receiving accurate local stimuli. In autumn and winter and early spring the males respond eagerly to other crayfish and conjugation is accomplished wherever possible. The entire series of conjugative actions seems preëxistent in the male to be awakened, at times, with explosive violence, on very slight stimulation. It is then carried out in its entirety whenever there is no mechanical obstacle.

Sex "recognition" exists, apparently, only in the sense that the male may carry out all the stages of conjugation if a female happens to be seized, but not if a male is seized. There is no sufficient evidence that the male recognizes the female as such, or as a whole. But the passive response of the female when seized makes the completion of the conjugation possible while the more effective resistance of the male when seized sooner breaks the series of conjugation acts.

The receptors of the male affected by male and female as objects seized appear to be only those that are stimulated by

actual mechanical contact. There is as yet no evidence that the light or chemical substances from the male and female act differently upon the males.

Crossing of species is not yet accomplished, though most of the phases of conjugation are carried on between species, in captivity.

The organs which are accurately adjusted during conjugation are of different values; some are ordinary and some specialized limbs; some are mere spines of the shell; one is a special invagination not represented in many other animals. Though some are old and some new they are all alike necessary at present for the meeting of sperm and egg.

It is by no means clear how any offered method of evolution can account for the assemblage of such constituents into their present working individuality.

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REACTIONS IN AMOEBA TO LIGHT¹

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TWO FIGURES

Verworn (1889, p. 40) studied the movements of *Amoeba limax* and *A. princeps* in a microspectrum and in a field of white light with sharp gradation of intensity and maintains that they crawled about from one color to another in the spectrum and from one intensity to another in the white light without any apparent reaction. In both cases the rays were perpendicular to the slide on which the amoebae were mounted. Davenport (1897, p. 186) also failed to obtain reactions in *Amoeba proteus* in white light under similar conditions, but he showed very clearly that these creatures orient and move from the source of illumination if they are exposed in a horizontal beam of intense light so arranged that no other light reaches them. This led him to conclude that the reactions in these organisms are due to the direction of the rays and not to difference of intensity. Rhumbler (1898) observed that sudden illumination causes a cessation in activity in *Amoeba verrucosa* feeding on filaments of *oscillaria*. Harrington and Leaning (1900) found that intense white or violet light thrown on an active specimen of *Amoeba proteus* causes the protoplasmic streaming to stop instantly. Red, on the other hand, they claim, causes an acceleration in movement, while green and yellow have very little effect. Engelmann contends (1879) that intense illumination causes the rhizopod *Pelomyxa* to contract. Ewart (1903) says that it causes a retardation or cessation in the protoplasmic streaming in many different plant cells. And both Baranetzski

¹ Contribution from the Laboratory of Experimental Zoölogy of Johns Hopkins University and the Biological Laboratory of Goucher College.

(1876) and Stahl (1884) state that if a portion of the plasmodium of certain myxomycetes is strongly illuminated the protoplasm in that portion stops and later tends to flow toward the part not illuminated. In view of all these facts it seems strange that Verworn and Davenport observed no reactions in amoebae as they crawled from a shadow into an intensely illuminated region.

My observations were all made on *Amoeba proteus* taken from a culture which Professor H. S. Jennings kindly put at my disposal. The amoebae came up in a hay infusion used in rearing paramecia. They were so abundant that hundreds were often found in a few drops taken from the surface of the debris where they collected, forming a dense grayish layer in many places. In all the observations numerous specimens were mounted in a clear solution under a large cover glass supported by a ring of vaseline so as to give them ample room for movement. It was found that they can be kept on a slide in this way in excellent condition for several days.

REACTIONS TO CHANGE IN LIGHT INTENSITY

By means of a mirror direct sunlight was flashed on specimens on a slide mounted in diffuse daylight and it was found that the increase of intensity caused all streaming to stop immediately regardless of the direction of the rays of light. There was however, no immediate contraction similar to that observed by Engelmann on *Pelomyxa*. After a few minutes' exposure new pseudopods usually formed at or near the posterior end and as these extended the old ones were withdrawn. It is an important fact that after the amoebae have been exposed to direct sunlight for a short time they appear to move as rapidly as they did in diffuse light and that if the intensity is gradually increased there is no apparent decrease in rate of movement, for it shows that the response described above is dependent primarily upon the time rate of change in light intensity and secondarily upon the amount of change.

These results agree in general with those of Harrington and Leaming and others mentioned above, but they do not bear on

the question of local reaction due to increase in illumination of a limited part of the organism and consequently throw no light on the factors involved in orientation. It was therefore necessary to study the effect of local increase in light intensity. This was done as follows: In a dark room a limited area of a luminous Welsbach mantle was focused on a slide containing amoebae by means of the mirror and the Abbe condenser attached to a microscope, so as to form an intensely illuminated and well-defined area about 0.5 mm. square. By carefully manipulating the mirror the intense light in the illuminated area could be suddenly flashed on any portion of an amoeba. It was found that an increase of intensity produced thus, usually caused a cessation in movement in the part stimulated, especially in case of newly formed pseudopods. But more convincing results were obtained by studying the reactions of specimens as they came in contact with the illuminated area in their random movements. Many observations were thus made on different individuals and it was found in nearly all instances that they stopped when they reached the light and proceeded in a different direction.

The details in the responses which resulted in a change in the direction of motion and thus kept the organism out of the intense light were essentially the same in all of the specimens observed. They are graphically recorded for a single individual in figure 1. By referring to this figure it will be seen that after one pseudopod came in contact with the illumination and was stopped, the amoeba did not at once proceed in the opposite direction so as to avoid the light but sent out other pseudopods at only a slight angle with the first apparently trying to get around the obstacle in this way. The character of the response did not change after the first pseudopod came in contact with the light nor did it change after the second and the third came in contact with it. But after the fourth became exposed the direction of motion was nearly reversed. This indicates that the reaction was modified, that the response to a given stimulus depends upon the preceding experience.

It is evident then judging from the reactions described that a sudden increase of intensity tends to inhibit the movement momentarily in *Amoeba* either locally or entirely and that this

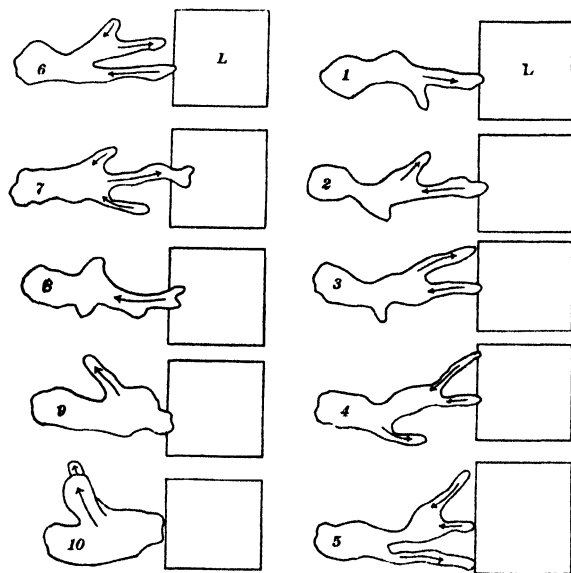


FIG. 1 Sketches representing the reactions of an amoeba proceeding toward an intense area of light the rays of which were perpendicular to the slide. *L*, field of light formed by focusing a limited section of a Welsbach mantle on the slide. 1-10, successive positions of an amoeba a little less than one-half minute apart. Arrows indicate direction of streaming in pseudopods.

response is dependent primarily upon the time rate in change of intensity. What bearing has this fact on the analysis of orientation?

ORIENTATION

The observations on orientation were made under a compound microscope situated in diffuse daylight without any screen around it. Mirrors were so arranged that two horizontal beams of direct sunlight were reflected on the stage at right angles to each other after passing through 8 cm. of water to eliminate the heat. Specimens exposed in one of these beams without any light from the sub-stage were found to direct their course in a general way from the source of light. In one instance, after a slide had been exposed for fifteen minutes, there were eleven specimens in one field of the low power, all but two of which were moving from the source of light. In another field there were twelve specimens; all but four of these were directed from the source of light. Of these four, two were proceeding at right angles to the rays and two were going toward the light. In still another field containing nine specimens, seven were negatively oriented, one positively and one at right angles to the rays. Orientation was however, not very precise in any of the specimens. Amoebae usually take a sort of zigzag course. Pseudopods were frequently seen to extend toward one side for some distance then stop as though they had been checked, after which new ones were ordinarily seen to extend on the opposite side for some distance and stop, etc.

The details in the process of orientation were observed as follows: a specimen which had oriented in one of the beams of light was selected, after which the light in this beam was intercepted and that in the other simultaneously turned on. The reaction of numerous specimens was observed in this way and the movements in several were recorded by means of camera sketches made at short intervals. A typical record is presented in figure 2. A majority of the specimens did not, however, orient as precisely and definitely as this one did. By referring to the figure it will be seen that the amoeba under observation gradually turned from the side most highly illuminated, sending out pseudopods only on the shaded side. What is the cause of this?

In changing the direction of the rays so that the amoeba became strongly illuminated from the side as previously described, the distribution of the light intensity on each pseudopod as well as that on the entire organism became changed. Some pseudopods became more highly illuminated; others became shaded; on some surfaces the intensity was increased; on others it was decreased. I could not however, be quite certain that this change of intensity inhibited movement in any pseudopod which had already begun to extend, although it often appeared as though it did. But by referring to the figure it will be seen that no new pseudopods formed on the illuminated side. It must be then that orientation in these organisms is due to the inhibition of the formation of new pseudopods on the more highly illuminated side. There was no evidence whatever that a pseudopod with one side more highly illuminated than the opposite became curved owing to difference in rate of movement on the two sides or unequal contraction or expansion.

It may now be asked, what is the cause of the inhibition in the formation of pseudopods on the more highly illuminated side and the consequent orientation? Is it the direction of the rays through the organism in accord with Davenport's conclusion (1897, pp. 187, 210, 211); or the difference of intensity on symmetrically located sensitive elements in accord with Loeb's theory of orientation (1906, pp. 130, 139); or is it changes of intensity? Are the different parts of an amoeba stimulated by light continuously in proportion to the absolute intensity on them as Loeb's theory demands or are they stimulated only when they get into such a position that changes of light intensity occur owing to the shadow of one part cast on another? All that can be said regarding these questions at present is that we are certain that a sudden change of light intensity may inhibit streaming movements; that after streaming is thus inhibited it may start again in course of a few minutes without any further change of intensity; that there is no evidence indicating that the direction of the rays through the tissue influences reactions; and that we are not certain what effect continued illumination without change of intensity may have on orientation since it is practically impos-

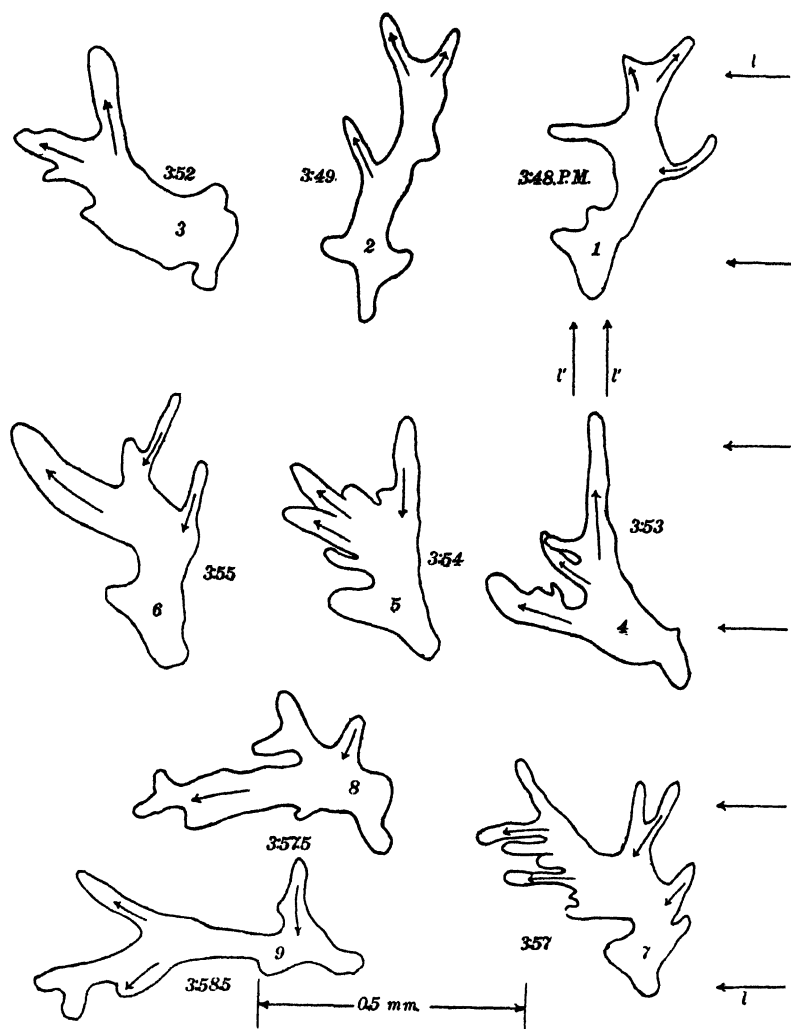


FIG. 2. Camera drawings representing different stages in the process of orientation in *Amoeba proteus*. 1, *Amoeba* oriented in light $l'l'$; 2-9 successive positions after exposure to light ll , time indicated in each. Arrows represent the direction of streaming of protoplasm in pseudopods. In those which do not contain arrows there was no noticeable streaming at the time the sketch was made. ll and $l'l'$, direction of light; mm, projected scale.

sible to subject an active amoeba to light without changes of intensity owing to the movement of the shadows of one part on others in the process of locomotion.

If orientation in *Amoeba* is due to changes of intensity as appears to be true, it is in principle the same as that in *Euglena* (Jennings, 1906, p. 138), *Volvox* (Mast, 1907, p. 153), and various other similar forms.

REACTIONS IN LIGHT DIFFERING IN WAVE LENGTH

In these observations the light waves were differentiated both by means of color filters and by means of a prism. The filters, kindly lent me by Dr. R. P. Cowles, were prepared and spectroscopically tested in the physical laboratory of Johns Hopkins University. The red was transparent from $620\mu\mu$ out, opaque from 450 to $590\mu\mu$ and faintly transparent from 380 to $450\mu\mu$. The blue was transparent from 430 to $490\mu\mu$ and from $690\mu\mu$ out, and opaque from 590 to $670\mu\mu$. The green was transparent from 380 to $400\mu\mu$, from 450 to $550\mu\mu$ and from $680\mu\mu$ out. It was opaque between 580 and $660\mu\mu$ and faintly transparent between 400 and $450\mu\mu$. The following table gives the relation between wave-length and color as used in this paper:

Red =	$630 - 760\mu\mu$
Orange =	$590 - 630\mu\mu$
Yellow =	$560 - 590\mu\mu$
Green =	$490 - 560\mu\mu$
Blue =	$430 - 490\mu\mu$
Violet =	$395 - 430\mu\mu$
Ultra violet =	$340 - 395\mu\mu$

The amoebae, mounted as described above, were studied under a magnification of about 150 diameters with very faint illumination from the mirror. A beam of direct sunlight which passed through 8 cm. of water was thrown on the slide at an angle of about 45 degrees with the stage. The organisms were exposed to light differing in color by intercepting the beam with the color filters. It was found that amoebae which moved actively in weak diffuse light ceased moving shortly after being suddenly exposed to

strong red light but soon began again. If they were now exposed to green the movement again ceased; the same was true for blue after green and for direct sunlight after blue. A change from direct sunlight to blue, blue to green, or green to red, produced no apparent effect. After being exposed to any color or any combination of colors for a short time the movement was resumed. In direct sunlight or in blue light it required longer than in green or red. As a matter of fact in these two colors, in the red in particular, there was no cessation of movement in some specimens, and only a slight decrease in others, while in still others the movement stopped entirely. In case of direct sunlight or blue, on the other hand, the movement stopped abruptly in nearly every specimen almost as soon as exposed. Similar but somewhat more detailed results were obtained in the spectrum.

In making the observations in the spectrum a horizontal beam of direct sunlight was passed through a vertical prism and thrown on the mirror below the stage of the microscope, from which it was reflected to the slide. By manipulating the mirror the amoebae on the slide could be suddenly subjected to light in any part of the spectrum, and the color to which they were exposed could be instantaneously changed.

The vertical slit in the opaque screen over the face of the prism was 2 mm. wide and the spectrum on the slide nearly 3 cm. long. There was consequently some overlapping rays in adjacent parts of the spectrum, but there was no intermingling of rays in distant parts. For example in the red there was some orange but no rays of shorter wave-lengths.

The amoebae were examined in daylight so faint that they could scarcely be seen. After a specimen which was active in this light had been selected, it was suddenly exposed to any desired part of the spectrum and the reaction noted.

Many observations were made on numerous individuals between 10 A.M. and 1 P.M. June 16 and 18. The sky was clear and the intensity of light consequently at a maximum, approximately 5000 candle meters. Without going into details with reference to reactions of individual specimens it may be stated that the effect of sudden exposure to red, yellow or violet after very faint diffuse

sunlight was essentially the same. There was in many specimens, a slight decrease in rate of movement, in some a momentary cessation, and in others no apparent reaction whatever. In the green the effect was similar to that in red, yellow and violet, only somewhat more marked. To obtain the effect described above it is necessary (1) to have amoebae in a certain condition, (2) to keep them in as low light intensity as possible before making the exposure, and (3) to use very intense light. When exposed in blue after having become active in any other color or in diffuse sunlight all movement stopped instantly in nearly all specimens observed. But there was no apparent contraction, the animals retained almost the exact form they had before the exposure. In some instances after remaining quiet a few seconds the streaming of the protoplasm in the anterior pseudopods slowly began again. In others new pseudopods were formed at the posterior end, and as these developed the old ones were slowly withdrawn. The movement ordinarily increased at such a rate that after 30 to 60 seconds it was again normal. If any other part of the spectrum was flashed on an amoeba which had become active in the blue there was no apparent reaction, but if such a specimen was exposed to direct sunlight it was clearly seen, in some instances, that the streaming ceased again.

The fact that the same reactions are obtained in the impure colors produced by means of filters and in the relatively pure colors of the spectrum is significant. It shows that a few foreign rays have no appreciable effect on the reactions of organisms which are not very sensitive to light. Moreover in case of *Amoeba* the response to the blue is so striking and that to the other colors so very slight that there can be no question as to the specific effect of the blue (430–490 $\mu\mu$). That is, the effect of different parts of the prismatic solar spectrum on *Amoeba* is not proportional to the energy contents, for the energy gradually increases as one proceeds from the violet toward the red end, whereas the region of maximum stimulation for *Amoeba* is in the blue, from which it decreases toward both ends. Nor is it proportional to the brightness as judged by the human eye, for the yellow is much brighter than any other part of the spectrum. In fact, under the conditions

of the experiment, one could hardly bear to look through the microscope when the yellow was reflected, while in the case of blue, the region of maximum stimulation for Amoeba, there was no unpleasant stimulation whatever to the eye.

Furthermore, it cannot be said without qualification that the actinic rays are most efficient in stimulating Amoeba, for in most photochemical reactions the violet and ultra violet are most active. As a matter of fact, however, the current idea that only the shorter waves of the spectrum are actinic is not supported by the results of recent experiments of Stobbe (1908) and others on photochemical reactions. Nor can it be said that the region of greatest efficiency in the solar spectrum is the same for all of the lower organisms, plants as well as animals, as maintained by Loeb (1905, p. 194) and Davenport (1897, p. 202), for Strasburger (1878) found the region of maximum stimulation for swarm spores in the violet; Wiesner (1879) found that for higher plants at the lower limit of the violet; Bert (1869), Lubbock (1882) and others found that for *Daphnia* and related forms in the green or yellow; Engelmann (1883) found that for *Bacterium photometricum* in the infra red, and Verworn (1899) maintained that all regions of the spectrum are equally active in stimulating *Oscillaria*.

All reactions to light are probably caused by or at least associated with chemical changes induced by the illumination. But the fact that different organisms are not equally stimulated by the different colors of the spectrum indicates that the chemical changes associated with the reactions are not the same in all organisms. This point is however, not irrevocably established, for while it is generally true that the reacting substances are different whenever photochemical reactions are caused by different colors, it is well known that the color which causes reactions between different compounds may be changed by the addition of certain substances which do not take part in the reactions. Thus it may be that the cause of difference in the response to different colors in organisms is not due to different photochemical substances within the organism, but to the presence of substances which do not take part in the reactions.

SUMMARY

1. A sudden and sharp increase of light intensity causes retardation or cessation of movement in *Amoeba proteus*. This effect may be local if the increase of intensity is local.

2. If the intensity remain constant for a few moments after a response, then the movement usually begins again. If the intensity is very gradually increased it produces no response, showing that the reaction to light in *Amoeba* is dependent primarily upon the rate of change of intensity.

3. *Amoeba proteus* is negative in strong light and orients fairly accurately. Orientation is brought about by the inhibition of the formation of pseudopods on the more highly illuminated side. This is probably due to local changes of intensity owing to the movement of the protoplasm and the resulting shadows of one part passing over others.

4. There is no evidence indicating that the direction of the rays through the tissue or absolute difference of intensity on different parts of the organism is functional excepting in so far as it may result in changes of intensity on the organism.

5. The blue (430 to 490 $\mu\mu$) in the solar prismatic spectrum is nearly as efficient in causing reactions in *Amoeba proteus* as white light. Violet, green, yellow and red are only very slightly active. Not all organisms, however, are most strongly stimulated in the blue of the solar spectrum. Some respond most definitely in the violet, others in the green and yellow, and still others in the red.

6. It is highly probable that different photochemical changes are associated with the reactions to light in different organisms.

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WHAT CONDITIONS INDUCE CONJUGATION IN PARAMECIUM?

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FOUR FIGURES

Why are Paramecia found to conjugate at certain periods while at other periods they multiply without conjugation? Work undertaken on the relation of conjugation to heredity and variation in these organisms has forced me to deal with this ancient problem. In the present paper I propose to give briefly, and without entering upon the history of the subject, an account of experiments and results upon this question. Discussion of the literature is reserved for a later and more extensive paper dealing with the effects of conjugation upon the organism and upon its life history.

The conditions producing conjugation have been sought in two different directions: on the one hand, in certain external conditions; on the other, in certain internal conditions.

The commonest view is perhaps the following: The life of these animals proceeds in cycles. There is a long period of multiplication without conjugation. During this period there is a gradual decrease in vitality, showing itself in degenerative changes and in a decrease in the rate of multiplication. Finally the animals can divide no more, and will die if conjugation or some equivalent process does not occur. Now they are ripe for conjugation. Conjugation is therefore due to a certain internal condition of maturity in the organism. For a presentation of this theory, see Calkins' recent valuable work "Protozoölogy" (1909).

DIFFERENT CONDITIONS FOR CONJUGATION IN DIFFERENT RACES

Experimentation shows that the question, *What conditions induce conjugation in Paramecium?* is wrongly placed; it must be subdivided. *The conditions for conjugation are different in different races.* Experimenters on this matter will reach quite different results in different cases, depending upon what races are used.

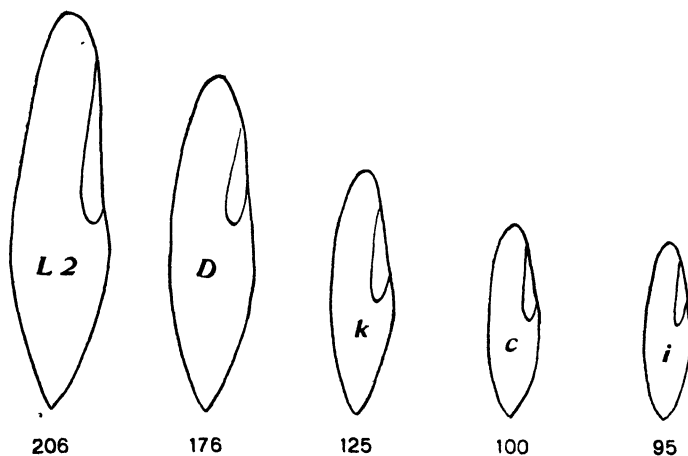


Fig. 1 Diagram showing the relative average lengths of the diverse races of *Paramecium* used in the present work. The actual mean length of each race is given in microns below the corresponding outlines. The outline marked *k* shows approximately the mean size of the races *g* and *C2* as well as of *k*.

In my recent paper on Heredity, Variation and Evolution in Protozoa (1909), I have demonstrated the existence of many races in *Paramecium*¹ differing constantly in average size. The relative sizes of a number of these races, including those with which I shall deal in the present paper, are shown in fig. 1. About ten different races or "pure lines", each derived originally

¹ The work of the present and earlier papers deals with the common infusoria that have been known under the name *Paramecium aurelia*, or *P. caudatum*, or under both names; to these belong the diverse races mentioned.

from a single individual, I have kept in the laboratory for periods varying from one and a half to three years. Some of these races have apparently never conjugated during the whole period. Others have conjugated once,—in some cases as an epidemic, in others only scattering individuals conjugating. Other races have conjugated two or three times, while certain races have conjugated many times, epidemics of conjugation occurring at brief intervals.

Observations on these races were not continuous, so that conjugation may have occurred at times when it was not observed. No stress is laid, therefore, upon the fact that certain races were not seen to conjugate at all. But the very great difference between the different races in the readiness to conjugate is clear, and is confirmed, as we shall see later, by precise experimentation. Certain races could be depended upon with certainty to conjugate whenever new food was added to the culture, while in other cases this never occurred. Some of the facts are as follows:

The large race *D*, used for the chief part of my study of inheritance and variation in size (see Jennings, 1909), was derived from a single individual taken April 10, 1907; it has now been in the laboratory, unmixed, for two years and ten months. During the first year it was under practically daily observation, and determined attempts were made to induce conjugation. It has never been observed to conjugate.

Now contrast the case of *D* with that of the culture *k*. This was not a pure line, but was derived from eight small pairs of conjugants of equal size, taken January 29, 1908. (As we shall see later, the fact that eight pairs were taken, instead of a single individual, does not affect the tendency to conjugate.) The culture *k* consisted of rather small individuals, having but two micronuclei. It was not observed so closely as *D*, but incidentally epidemics of conjugation were observed in it on the following dates: (1908) January 29; February 4; February 17-20; February 26; September 12; October 19; November 9; (1909) February 24-27; March 1-6; March 13; March 26; April 14; April 19; May 11; May 24; June 13; June 19; October 7-10; (1910) January 29; February 24.

Most of these twenty epidemics of conjugation occurred a few days after new hay had been added to the culture, though this procedure had no effect in producing conjugation in *D*, nor in many other strains.

The small strain *c*, likewise extensively used in the work reported in 1909, has been in the laboratory for the same length of time as *D* (two years and ten months). During this time it has shown two epidemics of conjugation, September 25, 1907, and March 8, 1909. Long continued systematic and varied experimentation with the object of getting it to conjugate at other times has been quite unsuccessful, although *k*, under precisely the same conditions, showed epidemics of conjugation every few weeks, or even at intervals of but a few days, as we have seen.

The very small race *i*, described in my paper of 1909, has shown, since it has been in the laboratory (two years and six months), on two occasions a few scattering conjugants. Attempts to produce epidemics of conjugation have been unsuccessful.

The very large race *L2* (two years in the laboratory) has shown two epidemics of conjugation.

The two races *g* and *C2*, of my paper of 1909, have resembled *k* in their readiness to conjugate, though the tendency is not quite so marked in these cases. But each has conjugated several times during the period (somewhat more than two years) that they have been in the laboratory. Both *g* and *C2* were derived from single individuals, the former taken November 13, 1907, the latter January 29, 1908.

In practical work in the laboratory the difference between the diverse races in respect to conjugation was most striking. Certain of the races could be depended on to give conjugants at short notice whenever such were desired, and epidemics of conjugation often occurred when there was no intention of producing them. With other races it was impossible to get conjugants, though they were much desired for other purposes.

These general observations were supplemented by precise experiments. Many of these experiments were undertaken for other purposes, but gave results on our present subject.

A large number of experiments were planned for the purpose of inducing conjugation between members of different races. Individuals of two races of very different size were placed together in the same cultures, and subjected to conditions such as might be expected to bring about conjugation. Owing to the differences in size it was easy to distinguish the different races while thus living together. In these mixtures the race *k* was usually one of the two constituents, since this race could always be depended on to conjugate. The race *k* was rather small in size—much smaller than *L2* or *D*, but much larger than *i*; so that one of these three races was usually mixed with *k*.

Almost invariably in such mixtures the individuals of *k* alone conjugated, those of the other race taking no part in the mating. This shows clearly that different conditions are required to induce conjugation in the different races. Some of the facts are as follows:

L2 and *k* were mixed October 8, 1908, with fresh hay. On November 6, *k* was conjugating, *L2* was not.

To this same mixture fresh hay was added February 18, 1909. On February 24, *k* was again conjugating, while *L2* was not.

The races *k* and *i* were mixed October 10, 1908; *k* conjugating October 19, *i* not conjugating.

Fresh hay added to this mixture February 18, 1909; *k* conjugating February 24, *i* not. Fresh hay added again February 24. New conjugation of *k*, March 1–3; *i* not conjugating. Another epidemic of conjugation in *k*, March 10.

Three new mixtures of *k* and *i* made March 1, 1909. On March 2 and 3, one contains conjugating *k* only; another *pairs of both k and i*. March 10, one of these mixtures has conjugating *k*, none of *i*.

On February 24, 1909, a mixture was made of *i* and *kb* (this consisting of the progeny of a single ex-conjugant of *k*, isolated November 9, 1908). Fresh hay was added at the same time. From March 2 to 8, *kb* was conjugating, while *i* was not. Again on March 26, *kb* conjugated in this mixture while *i* did not not.

Thus out of eleven epidemics of conjugation in mixtures of which *k* formed a part, the conjugation was confined to *k* in all

cases save one. In this one a few specimens of *i* conjugated at the same time as *k*. (In this case the two races did not cross; specimens of *i* conjugated only with other *i*; those of *k* only with other *k*.)

Somewhat similar facts were observed in certain mixtures of other races. Thus, in a mixture of $C2 + i$, made March 2, on the next day $C2$ was conjugating while *i* was not.

The differences in readiness to conjugate in the experiments given above are evidently not due, as might be supposed, to the fact that the race which refuses to conjugate has conjugated more recently than the other. On the contrary, it is certain that in all these cases the race *k* had conjugated since the other race, yet it conjugates anew when the other does not. In some of the cases indeed, as we have seen, *k* goes through two epidemics of conjugation during the course of the experiment, while the members of the other race do not conjugate at all,—demonstrating absolutely that the length of time since the last previous conjugation is not what determines the different behavior of the two races. The race *k* is constitutionally disposed to conjugate much more readily and frequently than the other races.

CONJUGATION AMONG PROGENY OF A SINGLE INDIVIDUAL

Is the greater disposition of *k* toward conjugation due to the fact that this culture was composed originally (as noted above) of eight similar pairs, instead of being derived from a single individual, as are the other races? Experiment showed clearly that this question is to be answered in the negative; conjugation is just as frequent when we use from this stock cultures derived entirely from a single individual. The experimental facts are as follows:

A single specimen, one member of a pair that had just conjugated, was isolated from *k*, November 9, 1908. This was called *kb*, and from it an extensive culture or "pure line" was derived. The cultures derived from this single ex-conjugant conjugated as readily and often as did the original culture *k*, when treated in the same manner. Thus, fresh hay was added to *kb* February 24,

1909, and the animals began to conjugate three days later, February 27 (continuing till March 6). At the same time a mixture of *kb* with *i* was made; in this the specimens of *kb* were conjugating March 2 to March 6. There was no conjugation March 7, 8 and 9, but on March 10, *kb* was conjugating freely again in both the pure culture and the mixture with *i*. Twenty-five pairs of progeny of *kb* were placed together March 10, and their progeny were conjugating anew about two weeks later (March 26) and again on May 11. There was likewise a new epidemic of conjugation in the original culture of *kb* on March 26. Not to enter into farther details, I may say that epidemics of conjugation were seen in the progeny of the single individual *kb* on the following dates: (1909) February 27 to March 6, March 10–13, March 26, April 19, May 11, May 19, May 24, June 13, June 19, October 10, (1910), January 29, February 24. (The long intervals between June and October and between October and January are due simply to lack of observations in those periods.)

It is thus clear that the race *k* has a remarkable tendency to conjugate readily, even though the progeny of only one individual are involved.

It was long held that the progeny of a single individual could not conjugate together without resulting in destruction. Calkins (1902, p. 175), however, observed conjugation in the progeny of a single individual without disastrous results, and the same thing had been observed for *Paramecium putrinum* by Bütschli (1876) and Joukowsky (1898). The general tendency of accurate work on the Protozoa has been to show that the importance attached to conjugation with animals of different descent was a mistake. In the conjugation of the race *kb*, above described, we have repeated conjugations within the progeny of a single individual, and the race is still flourishing. All of my races are indeed progeny of a single individual, and all save one have been seen to undergo repeated epidemics of conjugation,—in every case of course the progeny of a single individual conjugating together. The races are still flourishing, without indications of degeneration. I propose to discuss the physiological effects of conjugation in a separate paper. Here, however, it is perhaps of interest to ask the further

question, how often can re-conjugation occur in a single line of succeeding generations from a single individual?

On this question certain interesting data are given by my experiments with *kb*. This, as we have seen, is a single ex-conjugant, one of a pair of *k* that were conjugating November 9, 1908. From this, two diverse lines were cultivated with frequent re-conjugation, with history as follows:

First conjugation November 9: a single member of the pair multiplied till April 17 (with in the meantime many epidemics of conjugation among its progeny). Conjugation April 17: a single exconjugant (*ko*) isolated and allowed to multiply till May 19, when conjugation occurred among its progeny. A single member of a pair (*koc*) was again isolated after conjugation, and allowed to multiply. In the *fourth* generation, after but 16 individuals had been produced, conjugation again occurred among its progeny, giving after separation the individuals *koca*. This generation was unfortunately destroyed. The pedigree of *koca* may then be represented as in fig. 2.

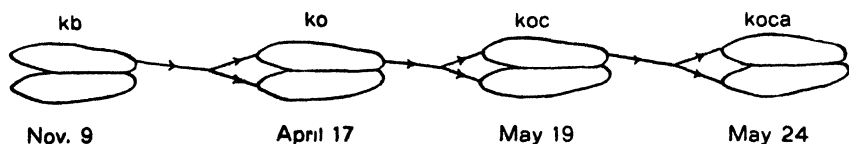


Fig. 2 Diagram showing the conjugations in the pedigree of *koca*. The fissions intervening between the conjugations are omitted. Each pair derived from a single member of the preceding pair.

We have thus four conjugations in series—the two members of any given pair being progeny of a single member of the preceding pair. Whether this inbreeding for four generations affects the stock in any way we shall discuss elsewhere.

In another case inbreeding has continued for five conjugations in series. From the conjugating pair of November 9, a single individual (*kb*) gives a numerous progeny, with frequent epidemics of conjugation among themselves. From one of these on March 9, twenty-five pairs were selected and placed together, producing numerous progeny (all of whose parents had thus certainly con-

jugated March 9). This lot was known as *kh*. These conjugated among themselves May 11, and a single member of one of the pairs was isolated, and allowed to multiply; it was designated *kha*. Among its progeny conjugation occurred again May 24,

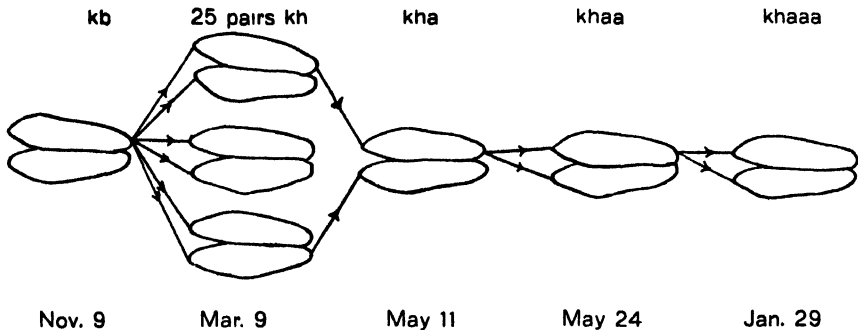


Fig. 3 Diagram showing the conjugations in the pedigree of *khaaa*. The fissions intervening between the conjugations are omitted.

and one member of a pair was again isolated after separation, receiving the designation *khaa*. This multiplied, lived through the summer and fall, underwent repeated epidemics of conjugation, and the chain of observation could be taken up again only in January, 1910. On January 29, a new conjugation occurred, and the single member of a pair *khaaa* was isolated. The culture *khaa* (fourth generation of inbreeding) is still flourishing; while prosperous beginnings of a culture are on hand descended from the single individual *khaaa* isolated from the fifth conjugation in series.²

The pedigree of the line just described may be represented by the diagram of fig. 3.

² This series has been continued, so that at the present time (Oct. 2, 1910) there have been nine conjugations in succession, all the conjugants at each mating being progeny of a single ex-conjugant from the previous mating. (Note added at correction of proof.)

INTERVAL BETWEEN CONJUGATIONS

In a given culture of the races *k* or *kb*, it is usually not difficult to cause by proper treatment epidemics of conjugation to appear at intervals of two or three weeks. I have never observed a period when this could not be done readily. The long intervals between some of the dates of observed conjugations, given above, are due merely to the fact that during those periods observation and experiment was not directed upon this matter.

If we isolate in each case a single member of a pair, and undertake to determine how long it may be before a new conjugation may occur among its progeny, we find the problem a little more difficult. It is necessary to cultivate the animals for a time with care, or they may be lost. As shown by the diagrams of pedigrees given above, the interval may be short; periods of one month; of two weeks, are shown in certain cases. With proper care, it is probable that the period could be brought regularly to little more than two weeks. In one case there is a period of but *five* days between the conjugation of the parent and that of the progeny (fig. 3). The ex-conjugant had divided but four times, into 16 progeny, when these progeny conjugated anew.

CONDITIONS UNDER WHICH CONJUGATION OCCURS

The great readiness to conjugate in the strain *k* gave an excellent opportunity for observation and experiment upon this point. In general, conjugation occurred in *k* under the following conditions: To a hay culture in which the infusion was getting "old," and in which the animals were rather thin and were not multiplying rapidly, a handful of fresh hay was added. The *Paramecia* at once began to get plump and to multiply. This is due to the fact that soluble substances from the hay diffuse into the water, increasing the growth of bacteria, on which the animals feed and apparently also directly increasing the growth of the *Paramecia*. Three or four days after the hay was added, conjugation began, the epidemic often lasting several days. At the time of conjugation it was noticeable that the animals had become thinner again,

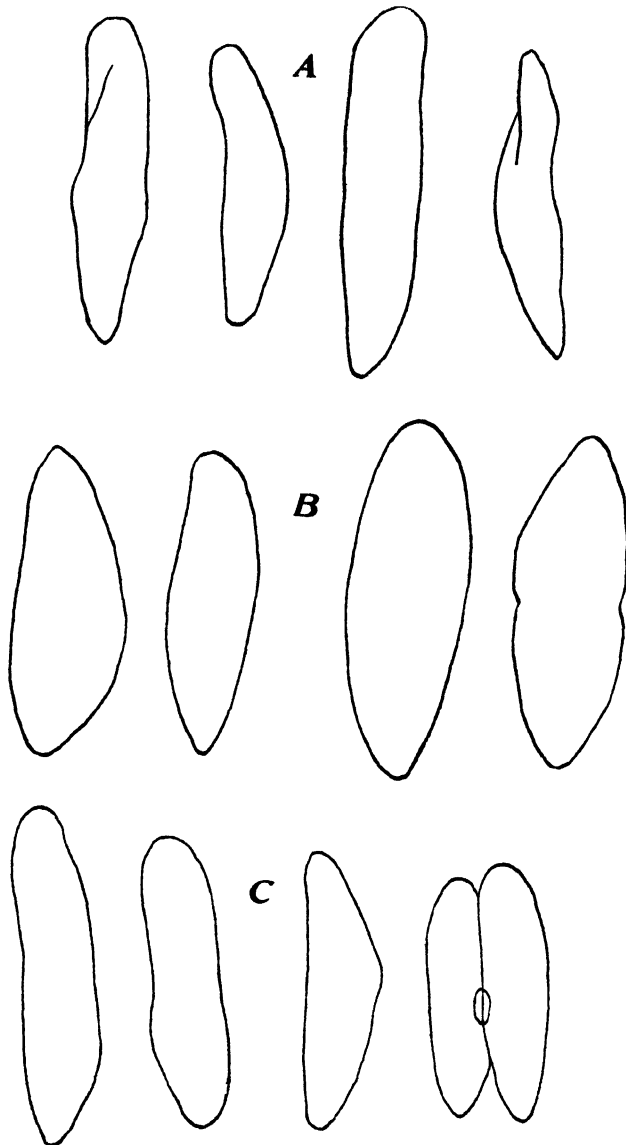


FIG. 4 Outlines (drawn with projection apparatus) showing characteristic changes in form and size as conjugation is brought on. Row A, specimens from the old culture, without fresh food, at the beginning of the experiment; no conjugation. B. Specimens from same lot after 24 hours in hay infusion; no conjugation. C, Same after three days in the same hay infusion, which is getting old; conjugation beginning.

and this continued during conjugation, multiplication at the same time almost ceasing. After conjugation the animals remain thin, and usually become fewer in number. After the infusorians have remained for some days in this condition, it sufficed to add hay anew in order to induce (after a few days) renewed conjugation.

The precise facts on this series of changes may be illustrated from an experiment in which the alterations in size were measured. On February 21, 1910, the hay culture of *kb* had become somewhat "old;" the animals were thin and rather few. This culture (which I have denominated on p. 287 as *khaa*) was derived from a single specimen of *k*, and consisted of individuals in which conjugation had taken place four times in a direct line, as illustrated in fig. 3. The last epidemic of conjugation had been January 29, less than a month before. From this culture I removed a number of samples, treating them as follows:

(a) A quantity of water from the old culture, along with many specimens, was placed in a watch glass in a moist chamber. The culture fluid being old, these individuals were allowed to hunger.

(b) A considerable number of individuals were transferred to fresh rich "standard" hay infusion (1 gram of Timothy hay, *Phleum pratense*, boiled for ten minutes in 100 cc. of tap water). This was kept in a watch glass in a moist chamber, along with (a). (Two samples were thus treated).

(c) In a third watch glass under the same conditions, individuals were placed in a mixture composed of half old culture fluid, half of the fresh standard infusion (two samples).

(d) The original large old culture was divided into two equal parts. To one of these a quantity of dry hay was added.

(e) The last culture consisted of the remaining half of the original old hay culture, to which nothing was added.

Thus of these five sets, two (*a* and *e*) were allowed to hunger, while three were placed in a fresh rich infusion. The latter began to grow and multiply at once, becoming plump and numerous. Two days later (February 23), conjugation began in the freshly fed sets *b* and *c*, while in the old cultures *a* and *e* there was no sign of it. In the culture *d*, to which new dry hay had been added, the animals grew less rapidly, since it requires time for the juices

of the hay to diffuse, so that the period of conjugation was not reached till February 25.

At intervals samples of each of the sets were removed and measured. As shown in my paper of 1909, abundant nutrition causes the animals to become much broader. At the same time, if rapid multiplication occurs, the animals may become a little shorter; whether this happens depends upon the relation of multiplication to growth. The breadth is therefore the dimension to examine in judging as to the nutritive condition. The measurements show clearly the relation of the conjugation period to growth and to hunger. They are as follows:

	NO MEASURED	LENGTH IN MICRONS	BREADTH IN MICRONS
Old culture; no fresh food	53	132 30 \pm 1 59	27 96 \pm 43
Same, after twenty-four hours in fresh hay infusion	41	127 56 \pm 1 19	41 37 \pm 55
Same, after three days in the new infusion; conjugation beginning	69	122 10 \pm 1.16	28 90 \pm 26

Thus, when the animals were placed in the fresh infusion, their breadth increased by 47.9 per cent in twenty-four hours. Later the breadth decreased again, and when it had, after three days, reached almost precisely the original breadth, conjugation began. Outlines of characteristic specimens at the three stages are shown in fig. 4.

The same relations are brought out if one examines the Paramecia of the large old culture, to which dry hay was added (*d* and *e*, above). Three days after the hay was added the animals were noticeably larger and thicker. Now a watch glass of the infusion, with many specimens, was removed to the moist chamber for twenty-four hours. At the end of that period the animals had decreased in size, and conjugation was beginning. The measurements are as follows:

	NO. MEASURED	LENGTH IN MICRONS	BREADTH IN MICRONS
Old culture, no fresh food	53	132.30 \pm 1.59	27.96 \pm .43
Same, three days after hay added; no conjugation yet	52	139.42 \pm 1.16	40.00 \pm .52
Same, one day later, in watch glass; conjugation beginning.	53	131.92 \pm 1.10	31.36 \pm .35

Here again conjugation followed upon a rapid decrease in the plumpness of the animals. But it is clear that *starvation* is not the cause of conjugation, for the animals that conjugated did not as a matter of fact (as the measurements show) become so thin as they were at the beginning of the experiment. Moreover, those left in the old infusion throughout the experiment (*a* and *e*, p. 290) continued to become steadily thinner, and at the end were much thinner than the specimens that conjugated. Yet these thinnest specimens never conjugated, because they were not subjected to the sudden change from rich to poor nutritive conditions.

Indeed, we can say, looking at the general course of events, that it is the addition of fresh food that finally results in conjugation; those parts of the culture to which fresh food is not added do not conjugate. The cause of conjugation is a *decline in the nutritive conditions after a period of exceptional richness that has induced rapid growth and multiplication*. To express this by saying that *hunger* induces conjugation is perhaps not incorrect, if we understand thereby relative hunger; hunger *after* satiety.

After conjugation has ceased, the animals usually decrease in numbers and remain very thin. But they can continue to exist in this condition for long periods. I regularly kept them through the summer vacation in this condition, no fresh material being added to the culture sometimes for a period of four months. During such times they existed in a sort of depressed condition, with extremely slow multiplication. I never observed conjugation in such periods, though it could readily be called forth by adding hay, as above described, or by placing them in a fresh infusion made by boiling hay in water.

Within a very short period after conjugation it is usually possible to induce a new conjugation by again adding hay. It is probable however that time must be given for loss of the plumpness and well-fed condition due to the previous feeding. Apparently three steps are required: (1) a thin, ill-fed condition, with little multiplication, followed by (1) abundant nutrition, causing plumpness, and rapid multiplication, and this followed by (3) a falling off in the nutrition, plumpness and multiplication. It is at the very beginning of this third period, when its effects are as yet hardly noticeable, that conjugation occurs. The interval between two successive conjugations appears to depend merely on the length of time required for inducing these stages in succession. As we have seen, intervals were observed in the case of *k*, varying from five days to two weeks; to a month or more.

CONDITIONS FAVORABLE TO CONJUGATION IN OTHER RACES

In the other races which I kept in the laboratory, epidemics of conjugation usually occurred under conditions similar to those observed for the strain *k*, but in most other races the epidemics occurred much more rarely, by no means taking place regularly whenever the conditions described were supplied. The difference between the strain *k* and other strains in this respect is well brought out in certain experiments that were planned for determining what external conditions would induce conjugation.

Thus, in the experiments with *kb* just described, I carried out at the same time a parallel series of experiments, identical in every detail, with individuals of the race *D*. This race had not conjugated for a long period, while *kb* had conjugated within a month. As we saw above, in *kb* conjugation occurred with absolute regularity as soon as the required fall in nutritive conditions was produced. Yet under the same treatment there was no conjugation in *D* at any time.

Again on June 9, 1909, I placed in five drops of a fresh rich standard infusion two individuals each of ten different strains, designated 82*a*, 21*a*, 21*b*, 16*a*, 16*b*. *kb*, *c*, *i*, *L2*, *D*. The first five strains had been isolated from a wild conjugating culture about a month

before, while the last five were races that had been cultivated one to three years in the laboratory. In this infusion the animals (each race of course in a separate vessel) multiplied rapidly. The infusion was changed frequently, all the cultures being treated in precisely the same way. On June 14, after all had multiplied extensively, the fluid was diluted to one-third its usual strength, and was not changed again. After a few days all began to become thinner and the division rate declined. On June 19 conjugation set in in *kb*, but in no other strain. The cultures were kept until the animals finally died from starvation.

This experiment brings out clearly the difference between the strain *k* and the other strains; cultivated under precisely the same conditions, the individuals belonging to *kb* conjugate, while the rest do not. Yet *kb* had undergone epidemics of conjugation more recently, before this experiment, than any of the other races involved.

It is clear therefore that different races of *Paramecium* differ extremely in their readiness to conjugate when favorable external conditions are supplied. Are the differences correlated with other noticeable differences in the races? As fig. 1 shows, some of the races are much larger than the others. Do the larger races, for example, conjugate less readily than the small ones? Or is the reverse the case? Examination shows that there is no constant relation between relative size and relative readiness to conjugate. The largest race, *L2*, conjugates rarely; the next largest, *D*, though long observed, has never been seen to conjugate. The smallest races, *c* and *i*, likewise conjugate only very rarely. The race *k*, which conjugates with such extraordinarily frequency, is of intermediate size, though belonging with the smaller rather than with the larger group. The other two races in which conjugation was not rare,—*g* and *C2*—were likewise races of intermediate size, differing little from *k* in this respect. The three races, *k*, *g* and *C2*, that conjugate most readily, have two micronuclei. But *c* and *i*, which conjugate but rarely, have likewise two micronuclei, while *L2* and *D*, also conjugating rarely, have but one.*

* For determination of the number of micronuclei in the different races, I am indebted to Dr. George T. Hargitt. A joint paper by Dr. Hargitt and the present author, on the characteristics of the diverse races, will appear shortly.

Whether, when conjugation does occur, the external conditions favoring it are the same for the other races as for *k* is a question of interest, but also of difficulty. In most cases where other races were seen to conjugate, the conditions were similar to those described above as favorable for the strain *k*. Usually there is a period of rapid multiplication, owing to abundant nutrition; this is checked, and an epidemic of conjugation results. With this the experience of other writers seems not inconsistent.

The fact that diverse races of *Paramecium* differ so greatly in respect to conjugation is evidently deserving of careful consideration in all experimental work on the life history of these organisms, and on the physiology of conjugation. Certain strains will be much more favorable for a given line of work than others, while investigators working with diverse strains are certain to reach discordant results.

Woodruff ('08, p. 526) makes the following remark: "I believe it is customary to regard conjugation as of far more frequent occurrence than it actually is in the life history of 'wild' individuals, because it is brought to the attention in laboratory cultures and 'hay infusions' which pass through a series of changes—changes which inevitably bring about conditions unfavorable to the continued reproduction of the organisms, and which are compensated for by conjugation." In this connection it may be of interest to note that I have repeatedly found conjugation occurring under "wild" conditions. This occurred in pools containing much decaying matter, where the water had largely evaporated. In such cases I found at times most of the individuals in conjugation. Examination showed that conjugation was in progress at the time animals were collected. Furthermore, conjugation occurs very commonly under the following conditions: A quantity of water, containing *Paramecia* with much vegetation, is brought in from a more or less stagnant pool. Nothing is added to this material, but the vegetation is allowed to begin decay in laboratory vessels. Almost invariably the *Paramecia*, if abundant, are found to begin conjugation inside of a week. There is of course no reason why the same thing should not occur in nature, when the same conditions are presented. The same conditions

are frequently presented as pools evaporate in summer,—and at such times, as we have seen above, observation shows that conjugation actually occurs. In all these cases conjugation takes place immediately after a period of rapid multiplication, when the nutritive conditions begin to decline—just as we have before set forth to be the case in the laboratory-bred cultures.

In strains that conjugate only very rarely, it is naturally difficult to study precisely the external conditions favorable to conjugation, and in strains that are never observed to conjugate, such as *D*, it is of course impossible. In one strain that was observed to conjugate but twice in nearly three years (the race *c*), the animals appeared at conjugation to be in a different condition from that observed in *k*. They were very thin, so as to appear starved. Apparently, the conjugation in this race comes on at a later stage in the disappearance of nutrition than is the case with *k*. But conjugation could by no means be induced at will by starvation in this race *c*, so that, with but two epidemics of conjugation, both appearing unexpectedly, it is difficult to judge as to the required conditions. It seems probable that the external conditions favorable to conjugation are in *c* somewhat different from those in *k*.

FACTORS DETERMINING CONJUGATION IN PARAMECIUM

Putting all the facts together, it is clear that the occurrence of conjugation (like most other functions of animals) depends partly on internal conditions, inherited from parent to progeny, partly upon external conditions. If we compare two races such as *k* and *L2*, we find that under the same conditions (and even when the two have lived for many generations under the same conditions), one race conjugates while the other does not. The difference is therefore due evidently to internal factors, to constitutional differences between the two races, inherited from parent to progeny. On the other hand, if we compare two cultures from the same race *k*, under different conditions, we find that one culture conjugates but the other does not. In this case the difference in behavior is evidently due to difference in external conditions;

the inherited tendency is the same in the two sets. What characterizes a given race then is an inherited method of responding to the environment,—this differing in the different races.

But even in the same race, the response to the same environmental conditions is not always the same. Under the same external conditions *L2* sometimes conjugates; at other times it does not. What are the internal differences that decide, within a given race, whether conjugation shall occur or not? In the case of *k* it is possible to form a more or less definite idea of what the determining internal conditions are. If the animals are thin and starved, they do not conjugate. If they are becoming well-fed, and increasing in vitality, they do not conjugate. If they have been well-fed, but have now begun to decrease, then they do conjugate. The differences between specimens that will, and others that will not, conjugate are thus more or less evident to the eye. But in such races as *L2*, *c* and *D*, where conjugation occurs only rarely, even though they are repeatedly put through the cycle of nutritive changes just described, the internal changes that initiate conjugation are much more difficult to define.

Is the change which brings on conjugation in the nature of decrepitude due to age; is it a consequence of the gradual running down of the machinery of growth and multiplication, worn out through a "cycle" of non-sexual reproduction, as is held by the commonly received theory? When we consider that in such strains as *k* conjugation may be repeated at intervals of two weeks or a month, or in some cases with an interval of but five days, and with only four generations between the two conjugations—it becomes difficult to believe that this hypothesis furnishes the correct explanation. A "cycle" in *k* might consist of but four generations! And when we recall further that Woodruff (1909) has kept *Paramecium* multiplying by fission for 1238 generations (26 months) without conjugation and without any indication of senile degeneration, and that Enriques (1907) has kept other infusoria for many hundreds of generations in the same way; when we recall also that I have kept the progeny of a single individual isolated from all others for three years without any indication of degeneration, doubt of the correctness of the theory of neces-

sary cyclical degeneration, remedied by conjugation, becomes still greater. But this question is wrapped up so closely with the problem of the effects of conjugation on the life history that it will be best to reserve our discussion of it for a later paper dealing with experiments on the subject just mentioned.

SUMMARY

1. The conditions determining conjugation differ greatly in different races of *Paramecium* (*aurelia* or *caudatum*). Some races conjugate frequently, and under conditions readily supplied in experimentation. Others, under the same conditions, conjugate very rarely or not at all.

2. The interval between conjugations may be very short, as in *k*, where epidemics of conjugation occurred, under proper conditions, at intervals of from two weeks to a month; in one case the interval between successive conjugations was but five days. In other races conjugation occurred only at intervals of a year or more. In one race *D*, though watched with care, conjugation has never been observed in a period of three years in the laboratory.

3. Frequent re-conjugation may occur among the progeny of a single individual. In *k* conjugation has been observed to occur in a certain direct line five times in succession,—both conjugants in each pair being descendants of a single member of the preceding pair. The descendants of an ex-conjugant re-conjugated in one case after but four divisions.

4. Conjugation occurred, in the races favorable for experimentation, not as a result of starvation, but at the beginning of a decline in the nutritive conditions, after a period of exceptional richness that has induced rapid multiplication. At the time of conjugation the animals are often in good condition, and multiplication may still be in progress.

In the races conjugating less readily, the external conditions favoring conjugation are probably somewhat diverse from those just set forth, yet of a similar general character.

5. The differences in different races in the matter of conjugation bear no simple relation to the relative size or other morphological characteristics of the races. The two largest and the two smallest races observed conjugate only rarely; the race that conjugates most frequently is intermediate in size. Among the races that conjugate but rarely are some with two micronuclei, some with but one. The race that conjugates most readily has two micronuclei.

6 The fact that in a given race conjugation may be repeated (in the same line of descent) at intervals of but five days to a month, and the fact that races derived from a single individual may live without degeneration for three years without admixture from outside, tend, along with the results of Woodruff, Enriques, and others, to weaken the theory that conjugation is to be considered the result of senile degeneration at the end of the life cycle.

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STUDIES UPON AMOEBA

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FORTY-FIVE FIGURES

I. ON THE LOCALIZATION OF THE EXCRETORY FUNCTION IN AMOEBA PROTEUS

Casual observation of the contractile vacuole shows in most species of *Amoeba* no evidence of its having any constant relation to any particular portion of the protoplasm. As the animal moves along with its characteristic flowing motion, the excretory vacuole flows along with the rest of the body, apparently very much as does any gastric vacuole or foecal particle. At more or less irregular intervals the excretory vacuole contracts and disappears, to reappear again after a time. It is a question of some interest whether, when the vacuole reappears, it reappears within the same portion of protoplasm which surrounded it before its last contraction. In most *Amoebae* the question is a difficult one to answer. But in some *Amoebae proteus*¹ which we have used in our laboratory in Oberlin this year and last, we find conditions that answer this question very definitely for us.²

¹ Either the species *proteus* includes very diverse individuals—diversent as to food habits, the condition of the crystals and plastids and plasma, and in the character of the excretory vacuole, or we should distinguish in the diverse types two or more species, or subspecies if preferred. The *Amoebae* here studied feed almost wholly upon bacteria, have numerous crystals, often contain much paraglycogen, and have spheroidal plastids that I have not yet studied. The granules around the contractile vacuole are better defined than those of other *Amoebae* I have studied.

² These *Amoebae* were obtained from Prof. J. H. Powers of the University of Nebraska.

Careful observation of the contractile vacuole shows that it is surrounded by a layer of granules of the same size and appearance as the microsomes of the general cytoplasm (fig. 1). When the vacuole is of moderate size these granules form a continuous layer, one granule thick, the adjacent granules nearly if not actually touching one another. When the excretory vacuole becomes very large the number of granules is insufficient to form a continuous layer, the layer of investing granules becoming irregularly interrupted, as one readily sees by focusing upon the surface of the vacuole (fig. 2). When the vacuole is small it lies in a mass of granules which surround it several deep on all sides (fig. 9).

Upon the contraction of the vacuole the granules formerly surrounding it remain, forming a very noticeable mass very different in appearance from the general cytoplasm (fig. 7). In the general cytoplasm the microsomes are loosely scattered. In the region from which the contractile vacuole has just disappeared in *systole*, the granules are as closely crowded as they can lie.

It is possible to keep this mass of granules under continuous observation for hours, and by so doing one sees that the contractile vacuole, when it reappears, appears in the midst of this same mass of granules, and that this is continuously the case. I have, in a number of instances, observed the conditions continuously for from two hours to three and three-quarters hours, and in no instance have I seen an excretory vacuole reappear elsewhere than among the same granules which surrounded it before its last contraction. There is, therefore, a constant relation between the excretory vacuole and a group of granules which surround it.

The details of *systole* and *diastole* are of some interest. Reference to the accompanying figures will show, without lengthy description, that after the vacuole has reached the full size, it crowds its way through the whole thickness of the *ectosarc* until at last it is separated from the circumnate water by only the pellicle (figs. 1, 3, 4, 5, 16, 17 and 18). This pellicle is soon forced outward into a considerable protuberance (fig. 4). Evidently it is quite tough and able to withstand considerable pressure. As the vacuole approaches the pellicle its granules first come into

contact with the pellicle (fig. 3). Soon the granules that lay between vacuole and pellicle are pressed aside and the contour of the vacuole and the pellicle come to co-incide (figs. 4, 17 and 18). After usually some minutes of increasing pressure upon the pellicle and of increasing protuberance, the pellicle finally breaks to form a minute pore³ and the contents of the vacuole exude so slowly that one has time to watch the change of form of the vacuole during its collapse (figs. 4, 5, 6 and 7). In two instances I was fortunate enough to see particles in the water outside the Amoeba pushed away by the current from the collapsing vacuole. Their movement began slowly and was not at any time a very violent one, indicating that the jet thrown out from the contractile vacuole is itself not very violent. In one of the two instances a slight vortex was produced by the jet from the vacuole.

Usually the very end of the contraction of the vacuole is slow. Very rarely a little of the contents of the vacuole may remain within the body and take part in the formation of the new vacuole.

After the contraction of the vacuole and its disappearance, its former position is, as already described, very clearly marked by the presence of the mass of granules which formerly enveloped the vacuole (fig. 7). Soon the ectosarc between these granules and the pellicle becomes as thick as in other regions of the body and one sees that the mass of granules lies in the endosarc but touching the inner portion of the ectosarc (figs. 7-12). The excretory vacuole of *Amoeba proteus* and probably of other species of *Amoeba* lies in the endosarc.⁴

Diastole takes place as has been described by others for *Amoeba* (figs. 7-19). From one to three small vacuoles first appear (fig. 8); two of these may unite, forming a larger vacuole (fig. 9); new small ones keep appearing in the immediate neighborhood (figs.

³ The pore is not itself visible, or at least I have not been fortunate enough to see it, but the manner of the collapse of the vacuole indicates a small opening.

⁴ It is customary in *Amoeba* to designate as ectosarc only the outer clear zone. The contractile vacuole does not lie in this zone. It does however lie in a less actively streaming zone attached to the inner surface of the clear zone. Doubtless the whole comparatively inactive outer zone, both its clear and its granular portions, is homologous with the ectosarc of ciliata.

10 and 15); from time to time these fuse with one another, or unite with the larger ones, till soon there are two or three moderate size vacuoles present (fig. 20); ultimately all fuse into one vacuole (fig. 16), which continues to enlarge until full size is reached (fig. 17), when it begins to push through the ectosarc as already described.

All these small vacuoles which appear and fuse lie among the granules in the mass referred to, or just outside them. There is a perfectly definite and constant relation between this mass of granules and the excretory vacuoles.

The Amoebae upon which these observations were made were for the most part very active. Their endosarc currents were very violent. It was often easy to see that the mass of granules surrounding the excretory vacuoles does not flow with the more rapid stream, but remains attached to the inner side of the more slowly moving ectosarc, and protrudes into the more rapid stream. This rapid stream tugs at the mass of granules, but in only one instance have I seen it produce any effect. Once the mass of granules was broken into two and the small group, surrounding a rather small vacuole, was carried off into the stream, while the rest of the granules, surrounding two moderate sized and two very small vacuoles, remained in position attached to the ectosarc. Unfortunately I followed the fate of the larger, more nearly stationary group of granules and do not know what became of the smaller group that flowed away. Infrequently, among these Amoebae, individuals with two contractile vacuoles are seen. One naturally imagines that this condition with two vacuoles may be due to a division of the mass of granules as just described, though I have not completely traced the origin of the second vacuole.

The mass of excretory granules lies, as described, attached to the inner surface of the ectosarc and does not flow with the endosarc currents. It is easy to determine that this connection with a particular region of the ectosarc is a constant one. The mass of excretory granules flows with the same rapidity as the adjacent ectosarc granules⁵ and it is plain that there is a constant associa-

⁵ These are much less evident than the endosarc granules, but can be observed.

tion between the excretory granules and this particular spot in the ectosarc. From this it is evident that the actual spot where the excretory pore forms from time to time when the vacuole contracts, is in an unchanging region in the ectosarc. To be sure this region flows from one position to another, but wherever it flows it retains its connection with the excretory granules and it continues from time to time to form a pore for the extrusion of the contents of the excretory vacuole. (See further discussion in Part II.)

One is naturally interested to know the nature of these granules which have such a close relation to the contractile vacuole, but my observations tell little of this. They resemble the ordinary cytomicrosomes in size and appearance; I have found as yet no stain which differentiates them from the cytomicrosomes; I have never seen any of these granules extruded with the liquid from the vacuole when it contracts. Yet the close association of this particular mass of granules with the excretory vacuole suggests that they have some functional relation to excretion. Comparison with the conditions in *Opalina* makes this seem somewhat more probable. I have elsewhere described the condition in *Opalina*.⁶ In the binucleated *Opalinae* there is an excretory vacuole, often of irregular shape, lying chiefly in the posterior end of the body, and opening to the exterior at the posterior tip of the body by a pore which, even when closed, is often indicated by a slight conical depression. There is almost always a posterior enlargement in the excretory vacuole, and around this vesicle are numerous granules closely resembling the cytomicrosomes, but staining a darker, less clear blue with Delafield's haematoxylin, and with rubin S and also with borax carmine showing a darker red. Granules exactly resembling those around the vesicle, except that they are some of them barely perceptibly larger, lie in the lumen of the vesicle itself. They are clearly merely some of the surrounding granules which have dropped into the vesicle. The posterior vesicle of the excretory vacuole contracts infre-

⁶ Metcalf: The Excretory Organs of *Opalina*. Parts I and II, in *Archiv f. Protistenkunde*, bd. x, 1907.

quently and at irregular intervals, and in its contraction many of the contained granules are thrown out and may afterward be found for a considerable time stuck to the posterior cilia of the *Opalina*. These granules seem to be connected functionally with excretion. The somewhat similar granules in *Amoeba*, if engaged in excretion (as seems to me altogether probable), are apparently less specialized than those in *Opalina*, for they show no special staining reactions differentiating them from the ordinary cytomicrosomes.

My colleague, Prof. Budington, has experimented with these same *Amoebae*, and he kindly allows me to incorporate here reference to some of his results of interest in connection with my observations. He found, when part of the body of an *Amoeba* is cut off and with it the contractile vacuole and its mass of granules, that the other portion of the *Amoeba* remains for a considerable time without a contractile vacuole, but later forms one. At first the new vacuole has few granules around it. The granules increase in number until (as I found), after about three hours, there are around the vacuole about one-third as many granules as around an ordinary vacuole. Probably in time the full number of granules would collect, but no individual has been observed long enough to prove this. The new vacuole will form in the divided *Amoeba* either when the nucleus is present or when it is absent, and it forms about as quickly in one case as in the other. The behavior of the new vacuoles formed in such divided *Amoebae* is the same as in normal *Amoebae*.

From these observations of Prof. Budington's it is evident that more than one portion, probably any portion, of the outer layer of the endosarc of an *Amoeba* may form a contractile vacuole, but it is also evident that when a contractile vacuole is once formed it soon associates with itself a mass of granules, and that this association persists, and that no portion of the protoplasm will ordinarily, if ever, form a new contractile vacuole so long as the already collected mass of granules associated with the old vacuole persists. Apparently there is just the beginning of specialization of cytomicrosomes in connection with excretion, and when there is a mass of these slightly specialized granules present, others

will not take on the function and enter into rivalry with them. Amoeba seems thus to show real though very slight physiological differentiation on the part of certain cytomicrosomes in connection with excretion. Opalina shows a higher differentiation.

What causes the granules to collect about the contractile vacuole? The results of Prof. Budington's operations upon Amoeba show that a new vacuole in an Amoeba fragment appears first and that only gradually the granules collect about it. The granules are not essential to the functional vacuole. All the appearances seem to indicate that the excretory granules come from the ordinary cytomicrosomes and are but very slightly modified functionally from the general cytomicrosomes. May it not well be that the repeated contraction and expansion of the vacuole, with the somewhat intermittent flow of liquid from the cytoplasm to the vacuole, cause some of the cytomicrosomes to move with the currents toward the vacuole and thus to form the group of granules described? Of course all the cytomicrosomes, like all the nuclear and extra-nuclear protoplasm, are engaged in metabolism and share in excretion. Apparently the special group of granules around the vacuole is slightly modified functionally, else why should the presence of a contractile vacuole and its granules in one region of the body prevent the formation of other contractile vacuoles in other regions of the body, since Prof. Budington's operations show that probably any region of the body is capable of forming a contractile vacuole.

I have not as yet had favorable material for studying these phenomena in other species of Amoeba. The species I had for study seems to be a form of Amoeba proteus. The individuals were very large and quite typical Amoeba proteus, except that their diet was almost exclusively bacteria, which is not ordinarily true of Amoeba proteus, and excepting also the condition of the excretory granules which are not so clearly marked in most Amoebae of this species.

II. A NEW SPECIES OF AMOEBA, PARASITIC IN TADPOLES

For the sake of comparing the excretory vacuole of this new *Amoeba* with that described in chapter I for *Amoeba proteus*, I include here an incomplete description of this form. I cannot give a complete description because by mistake my notes, drawings and measurements of these animals were left at my summer laboratory on the coast of Maine, and cannot now be obtained.

This minute *Amoeba*, parasitic in the recta of tadpoles of *Bufo cinereus*, *Rana esculenta* and *Bombinator pachypus*—all from Würzburg, Bavaria—is spatula-shaped with the posterior end mammillated much as in large *Amoeba proteus* (fig. 24). At its extreme anterior end, the broader end, is a large vacuole completely filling this part of the body. Close pressed against the posterior face of this vacuole lies the nucleus. The nucleus and vacuole are both slightly flattened on their contiguous surfaces, doubtless by pressure. The nucleus is not of the ordinary *proteus* type, but is of the type characteristic of the *Entamoebae* and most other minute *Amoebae*. Just inside its nuclear membrane are numerous chromatin granules. It has a well defined caryosome, with chromatin granules just inside the caryosome membrane. A caryole is present. A few irregularly scattered chromatin granules are found in both caryosome and nucleus proper, not abutting on the membrane.

One point of interest in this connection is that I have never seen the anterior vacuole contract, though I have studied several dozen individuals at different times and for considerable periods at a time. The vacuole rarely, if ever, contracts when studied on the slide, either in undiluted fluid from the rectum, or in normal salt solution containing all the rectal contents. Even if it seldom contracts, such a vacuole must be of assistance in excretion, since its contents are separated from the circumambient water by merely the thinnest film of protoplasm.

The constant relation in position between nucleus and vacuole in this species is also of interest. I have shown for the binucleated *Opalinae* that when the excretory tubule (vacuole) is well developed, its anterior portion lies touching the nuclei, and is

even bent around the nuclei so as partially to envelop them. In *Hoplitophrya* also the tubular excretory vacuole lies along the side of the meganucleus. The intracellular tubules of some metazoan cells similarly come into close relation to the nucleus.⁷ Do not these phenomena suggest that there is some functional value in such close association of nucleus and excretory vacuole?

We have seen in Part I of these studies that the contractile vacuole and its surrounding granules in *Amoeba proteus* remain in constant juxtaposition with a particular spot in the ectosarc, and that it is in the pellicle over this spot that the minute temporary excretory pore appears in the repeated contractions of the vacuole. In the *Amoeba* we are now discussing the vacuole rarely, if ever, contracts. This vacuole has a constant position in the very anterior end of the body. But this *Amoeba* moves in the manner described by Jennings and which is characteristic of *A. limax*, *A. blattae*, *A. verrucosa*, *A. proteus* and others, namely, by rolling forward, and not, as in most *Entamoebae*, by the explosive or eruptive formation of pseudopodia.⁸ In this rolling type of progression any given spot upon the ectosarc of the upper surface is constantly moving forward till it reaches the anterior end of the body, when it moves downward to the lower surface, the animal continuing to roll forward over it until this same portion of ectosarc comes to be posterior and then again upon the upper surface. If the excretory vacuole were to retain a constant relation to a particular portion of the ectosarc it would have to rotate with the ectosarc, being first anterior, then ventral, then posterior, then dorsal, and so on. But the vacuole does not so rotate, remaining instead always at the extreme anterior end of the body. The vacuole, therefore, in this species, can have no con-

⁷ I would not urge strongly this interpretation of the intracellular tubules of nerve cells and other metazoan cells as excretory.

⁸ I have not studied the locomotion of this species of *Amoeba* with the closest scrutiny of each detail. I have not traced the course of any particular spot on the ectosarc to demonstrate that this *Amoeba* has the type of locomotion described by Jennings, but even without this demonstration the appearance is so exactly like that in *A. limax* and so entirely different from that of *Entamoebae*; that one cannot doubt the locomotion to be of the rolling type. The ectosarc rolls; the endosarc streams forward.

stan: relation to any particular portion of the ectosarc. May there not be a correlation between this fact and the fact that this vacuole rarely, if ever, contracts? In the *Amoeba proteus* described in Part I, the contractile vacuole gradually pushes outward through that portion of the ectosarc which especially belongs to it, until it reaches the pellicle. It causes this pellicle to bulge outward, until finally, after a number of minutes, this bit of stretched pellicle ruptures and allows the contents of the vacuole to exude. It requires considerable and continued pressure upon the ectosarc and pellicle to secure an aperture for the discharge of the contents of the vacuole. In the rapidly flowing parasitic *Amoeba*, we are now describing, no one region of ectosarc and pellicle remains long enough in contact with the vacuole for the vacuole to succeed in pressing its way through to the exterior. That there is pressure upon the nucleus and vacuole, urging them forward, is apparently shown by the fact that that face of the nucleus and that face of the vacuole which touch one another are both slightly flattened as if from pressure. This pressure, caused doubtless by the forward currents of the endosarc, is insufficient to force the vacuole through the constantly changing portion of ectosarc and pellicle in front of it.

When I again have access to my notes upon this species I shall publish a somewhat fuller description, giving measurements. I shall then name this species *Amoeba* (*Entamoeba*)⁹ *currens* because of this very active locomotion, more rapid than in even the most active *A. proteus* or *A. limax*, and far more rapid than in *Entamoeba coli* or *E. tetragena*.

⁹ I do not distinguish between *Amoeba* and *Entamoeba*, for I have found, in what I take to be minute *Amoeba proteus*, nuclei of exactly the *Entamoeba* type. The parasitic habit is not sufficient for generic distinction, and no more is the fact that some species of *Amoeba* have a vegetative stage when their bodies are very large and their nuclei do not show caryosomes and caryoles.

III. THE LIFE CYCLE IN AMOEBA

A. *Amoeba proteus* (?)

At the mid-winter meeting of the American Society of Zoölogists in 1907, a paper of mine was read, describing briefly the formation of flagellospores in *Amoeba*, and their behavior in sexual reproduction. This paper was not published at that time. The observations were first made at the Woman's College of Baltimore in the spring of 1905, and would have been published more promptly had I not been somewhat uncertain whether the phenomena belonged to the life history of *Amoeba* or to that of a parasite of *Amoeba*. Further study convinces me that we have in these phenomena a true sexual process of *Amoeba* itself. The work is not yet completed, but I will no longer delay publishing an outline of the observations.

In the valley of the Patapsco river, near Baltimore, there is a small, never-drying spring, in which, and in whose outflowing streamlet, one rarely fails to find very abundant *Amoeba proteus*. In the spring of the year 1905 I collected a large quantity of mud and leaves from this spring and stream and placed it in an aquarium, intending to divide the material later into several parts, so as not to overstock the aquaria. This, however, was neglected and after about a week the aquarium was very foul and evil smelling. Casually examining the scum that covered its surface, my attention was drawn to certain very peculiar *Amoebae* of exceedingly minute size, that occurred in countless millions all through this surface scum, the whole field of the microscope being filled with them.¹⁰

Perhaps the most strikingly interesting forms were such as the one drawn in fig. 34 (magnified 1700 diameters). In such individuals the major part of the body was usually in a more or less globular mass, from one or more places upon which a few pseudopodia, composed mostly of ectosarc, protruded. These pseudopo-

¹⁰ I have since found these *Amoebae* equally abundant in material collected from each of half a dozen other localities.

dia were generally rather active, the Amoebae moving about with sufficient rapidity to render camera drawing a little difficult.¹¹

Upon the rounded portion of the body in these Amoebae, one observed, in different individuals, from six to thirty globular protuberances ("gemmules") each containing from eight to a dozen or fifteen little highly refractive spherules, doubtless nutritive plastids similar to those so common in Foraminifera, Flagellata and Ciliata.

With the aid of some of my students, individual Amoebae of this sort were kept under continuous observation, once for forty-eight hours, and three times for a period of thirty-six hours, while other individuals were observed at intervals of a few hours, each one for more than a week at a time. No gemmule was ever seen to detach itself from an Amoeba. On the other hand many Amoebae were found, from which gemmules had recently been set free. Many of these Amoebae were wholly or partially surrounded by a thick degenerated or degenerating cyst, while others showed no trace of an enveloping cyst. From the fact that so many hundreds of thousands of these Amoebae were seen with the freed gemmules still lying near them, it is evident that the gemmules, after being freed, often, if not always, remain inactive for a considerable time.

All through the liquid were countless millions of biflagellated organisms (Cercomonads) of the same type as the gemmules attached to the Amoebae, as just described, and showing the same highly refractive plastids within them (figs. 35 a-h). Often also a minute contractile vacuole was visible. These Cercomonads swim but slowly by feeble movements of the flagella. Frequently one sees them draw in their flagella and become amoeboid. Again, after a time, they may resume the flagellate condition, often one flagellum (the anterior one) forming before the other. Sometimes some of these amoeboid individuals attach themselves to the cover glass, flattening out upon it and showing grotesque shapes as they crawl slowly about (fig. 37 a-f). The resemblance of these amoeboid and biflagellate forms to the gemmules already

¹¹ All the figures given are from camera drawings.

described is so exact as to leave no doubt that the former are derived from the latter. They are amoeboid-flagellospores of the Amoebae.

The flagellospores occasionally divide by binary fission (figs. 36 and 39), but always, so far as I have observed, first drawing in their flagella. One cannot therefore say what constant relation, if any, exists between the plane of division and the position of the flagella. This binary fission is not very common. One sometimes searches for hours without finding a dividing individual. Again several may be found in one field of the microscope.

One not infrequently sees two flagellate individuals come into contact (figs. 40, 41 and 42). Sometimes they seem to pay no attention to one another. In other instances they apply themselves closely to one another by their anterior ends, drawing in their flagella, and while still clinging together anteriorly constantly, though slowly, changing the form of the posterior ends of their bodies (fig. 41). In a considerable majority of the cases observed, the animals separated after a period of contact varying from two or three minutes to half an hour. I have but once followed the process of copulation through from the beginning to its completion. In this instance the biflagellate isogametes met, pulled in their flagella, and became fused by their non-granular anterior ends (fig. 42). Gradually the bodies became completely fused and the zygote, a typical *Amoeba* of the *blattae* type (fig. 42, e), crawled slowly away. It soon, however, changed its form, developing pointed pseudopodia (fig. 42 f). Its behavior was not followed further. I have several times, probably a dozen times, observed what was probably the completion of copulation, already begun before these animals attracted attention. The animals have been studied at all hours of the day and night and at no time is conjugation frequent. In one field of vision, with an immersion lens, hundreds of these minute flagellates may be present. It seems strange that instances of copulation are not more frequent.

The question of the fate of the *Amoeba* after its gemmules are set free is an interesting one which I cannot answer. When these

Amoebae are bred in pure cultures on agar plates, gemmulation occurs only in individuals that are encysted,¹² and in these cases the body of the Amoeba from which gemmules have been freed disintegrates. One sometimes sees in the aquaria similar disintegrated bodies within the cysts which contain gemmules, but more often the body of the Amoeba, whether in a cyst or not, does not immediately degenerate after freeing its gemmules. Its ultimate fate I do not know.

The nuclear phenomena have not yet been satisfactorily worked out, and I reserve for a future communication their description. I may, however, say that there is fragmentation of the nucleus in the parent Amoeba, and that each flagellogamete contains a single nucleus.

At first sight it seems not improbable that these phenomena are to be interpreted as the growth and emergence of parasites from the Amoeba, but more careful observation shows that this cannot well be. In the early stages of the internal phenomena, preceding gemmulation, one sees in the body of the Amoeba many scattered refractive plastids not at all aggregated into groups (figs. 27, 28, 29, 30). In other, evidently later, stages some of these plastids are found aggregated into groups surrounding small nuclei (fig. 31). Other, and evidently still later, stages show that these nuclei, each one with its surrounding plastids, have moved to the surface of the body and there protrude, as shown in figs. 32, 34. Sometimes many refractive plastids are left in the body of the Amoeba after all its gemmules are freed. Often only very few plastids remain, sometimes none are seen. From their arrangement in the body of the Amoeba, it is clear that the refractive plastids are scattered through the protoplasm of the Amoeba itself and are not grouped in the bodies of any parasites within the body of the Amoeba.

The fact that biflagellate forms of the *Cercomonas* type are a stage in the life history of Amoeba is of no little interest. It is in harmony with the increasing belief that the flagellate type is more primitive than the Amoeboid.

¹² All the Amoebae on agar plate cultures encyst after a few days.

What is the full life cycle of Amoeba? Doubtless some species have a more complex cycle than others. Some of the earth Amoebae, so far as we know, reproduce only by binary fission, copulation occurring between nearly full sized individuals and no gametospores being formed. The Entamoebae (*coli*, *histolytica* and *tetragena*) have the same type of life cycle as the earth Amoeba. Schultze showed that *Amoebae proteus*, when in the full grown stage, reproduces by binary fission (fig. 25 a). Scheel¹³ described the formation of small endamoebospores to the number of six hundred or so to a cyst (fig. 25, d-f). These amoebospores were not seen to copulate, and there is no reason to believe that they do so. Calkins¹⁴ has described in large sized *Amoeba proteus* the presence of numerous neuclei (?) which he thought probably would give rise to the nuclei of gametes. It seems, however, not impossible that what Calkins observed were parasites within the Amoebae. I am inclined to believe that the gemmulating Amoebae described in this paper are *Amoebae proteus*.

Are there three or more sorts of reproduction in the life cycle of *Amoeba proteus*? I doubt if this question will ever be answered by isolating one or a few individual *Amoebae proteus* and making them go through their whole life cycle under observation. The whole life cycle probably requires a year or more for its completion, and it is doubtful if *Amoebae* can be reared for so long a time, and the environmental conditions so changed at the proper times, as to afford the necessary stimuli to instigate each of the several sorts of reproduction that may belong in the one life cycle. There is no conclusive proof that the *Amoebae* described in this paper are *Amoebae proteus*, but the small "radiosa" forms (fig. 26) look like young *Amoebae proteus*, and *Amoeba proteus* of the typical larger sort was very abundant in the material before it became foul. The evidence seems to make it probable that the phenomena described belong in the life cycle of *Amoeba proteus*.

¹³ Beiträge zur Fortpflanzung der Amöben, *Festschrift zum siebenzigsten Geburtstag v. Kupffer's*.

¹⁴ Evidences of a sexual cycle in the life history of *Amoeba proteus*, in *Archiv. f. Protistenskunde*, bd. v, 1904; and The fertilization of *Amoeba proteus*, in *Biological Bulletin*, vol. xiii, 1907.

and that this life cycle is a complicated one. The phenomena observed by Calkins cannot be properly judged without further study.

B. Amoeba sp. (?)

In the same aquaria in which were found the Amoebae described in section A of this part of this paper, and also in other foul aquaria stocked from other localities, one sees very numerous Amoebae of a different species (figs. 43 and 44), which also have external gemmules. They are smaller than the forms just described, and have much smaller gemmules, each containing but a single refractive spherule. Most of these gemmulating Amoebae are encysted, but some are found with no surrounding cyst. The gemmules when freed give rise to excessively minute Amoebae with no marked pseudopodia, resembling in form short and stocky *A. limax*. Further description and discussion will be reserved for a future paper. The gemmulating Amoebae of this smaller sort somewhat resemble individuals described by Penard¹⁵ as *A. radiosa* var. *gemmifera* and their manner of reproduction is similar to that described by Mercier for *Amoeba blattae*.¹⁶

C. A European Amoeba with reticulate Amœbosporos

In Würzburg I found gemmulating Amoebae in the surface scum of foul aquaria, stocked from the ponds in the garden of the Zoölogical Institute. Neither these Amoebae nor their gemmules contained refractive plastids. The gemmules, when freed, first became Amoeboid, but soon developed very long and exceedingly delicate reticulate pseudopodia, whose branches were so fine as to be on the very border of visibility with a Zeiss 2 mm. apochromatic objective and illumination from a gas mantle (fig. 45). These reticulate amœbosporos unite into groups of from two to five by means of their branching pseudopodia, but true copulation was not observed.¹⁷

¹⁵ Notice sur les Rhizopodes der Spitzburg, in *Archiv f. Protistenkunde*, Bd. ii, 1903, see p. 245.

¹⁶ Le cycle évolutif d'*Amoeba blattae* Bütschli, *Archiv f. Protistenkunde*, Bd. 16, 1909.

¹⁷ These were studied only a few times and but few hours at a time. My failure to observe copulation is therefore no indication that it does not occur.

EXPLANATION OF FIGURES

EXPLANATION OF FIGURES

Figures showing successive conditions of the contractile vacuole in one individual of *Amoeba proteus*. All are camera drawings of optical sections, except fig. 2, which is a view of the granules on the upper surface of the contractile vacuole, and all are magnified 337 diameters.

The irregular contours of the original, very rapid sketches, made from the living changing structures, have not been altered in the final drawings. The vacuoles were in reality spherical except when flattened on one side by pressure against the pellicle or when in contraction.

1 A typical full-sized contractile vacuole.

2 The granules over part of the upper surface of a large vacuole, showing that they do not make an uninterrupted layer over a very large vacuole as they do over one of moderate size.

3-23. Are consecutive stages in the expansion and contraction of the vacuole (two and one-half cycles).

3 At 2:50½ p.m. The vacuole has nearly reached its fullest size, has pushed through the ectosarc and now lies with its granules touching the pellicle.

4 At 2:52 p.m. The vacuole is larger, its granules are pushed to the sides until the contour of the vacuole coincides with the pellicle, which is pushed outward.

5 At 2:53 p.m. The vacuole has begun to contract.

6 At 2:53½ p.m. The vacuole has nearly disappeared.

7 At 2:55 p.m. The vacuole has wholly disappeared except for the faintest trace among the granules. The granules which formerly surrounded the vacuole as a single layer now form a considerable mass.

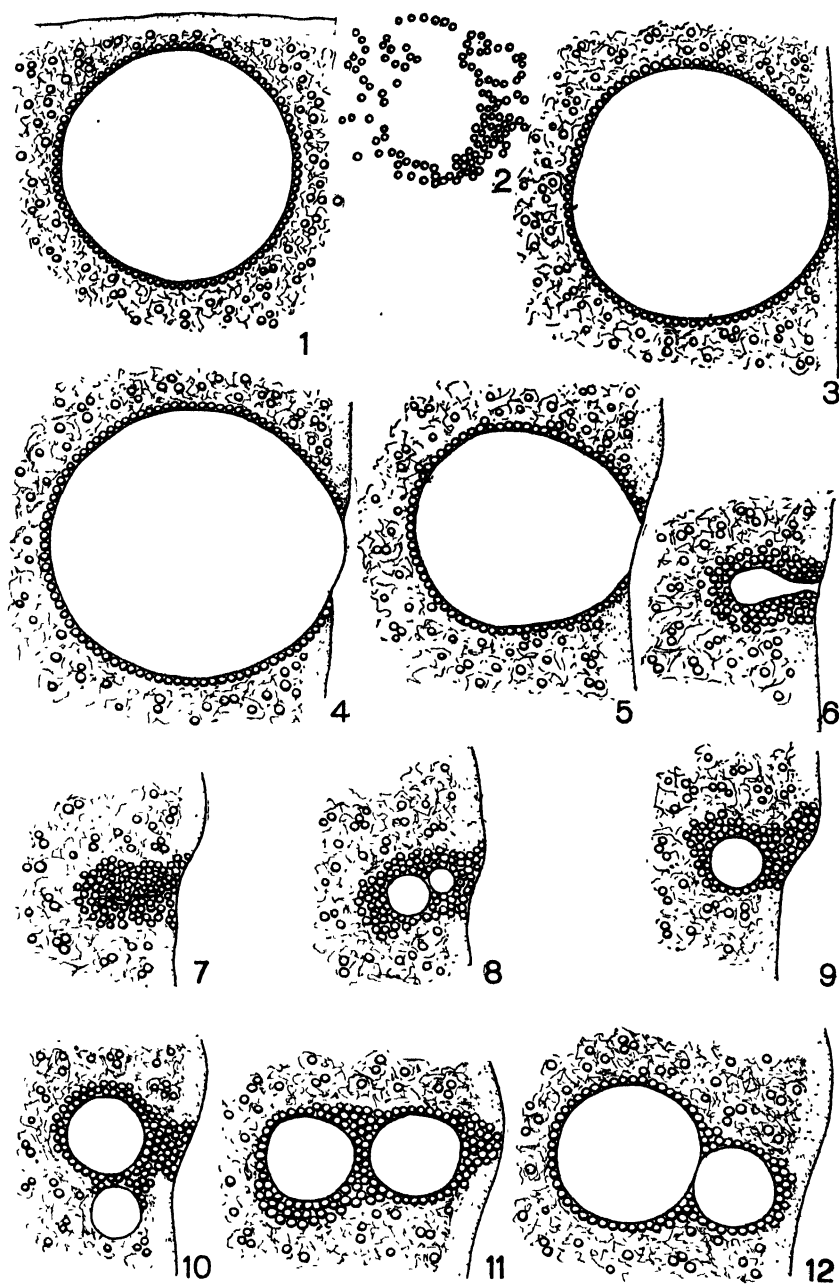
8 At 2:55½ p.m. Two small vacuoles have appeared in the midst of the mass of granules.

9 At 2:56 p.m. The two vacuoles have united into one.

10 At 2:57 p.m. This fusion vacuole is now larger, and another small vacuole is formed on the outer edge of the mass of granules.

11 At 2:57½ p.m. The first vacuole is still larger and the second has been taken into the mass of granules and has rapidly enlarged to the size of its neighbor.

12 At 2:58½ p.m. The two vacuoles shown in the last figure have now united and another one has formed nearer the ectosarc.



EXPLANATION OF FIGURES

13 At 2:58½ p.m. These two vacuoles are about to fuse.

14 At 2:59½ p.m. The two vacuoles have fused and the resultant single vacuole has somewhat enlarged.

15 At ½ minute past three. Another very small vacuole has appeared among the granules.

16 At 3:08 p.m. The vacuole has reached nearly full size. It is now surrounded by a single layer of granules. The four small vacuoles in the cytoplasm near the contractile vacuole did not fuse with the contractile vacuole, but remained unmodified near it. They were not drawn in the next three figures, though they were present.

17 At 3:16 p.m. The contractile vacuole has enlarged a little, has pushed through most of the ectosarc and is almost touching the pellicle. Its granules have been pushed aside from the point where the vacuole is to meet the pellicle.

18 At 3:17 p.m. The contour of the vacuole and the pellicle now coincide.

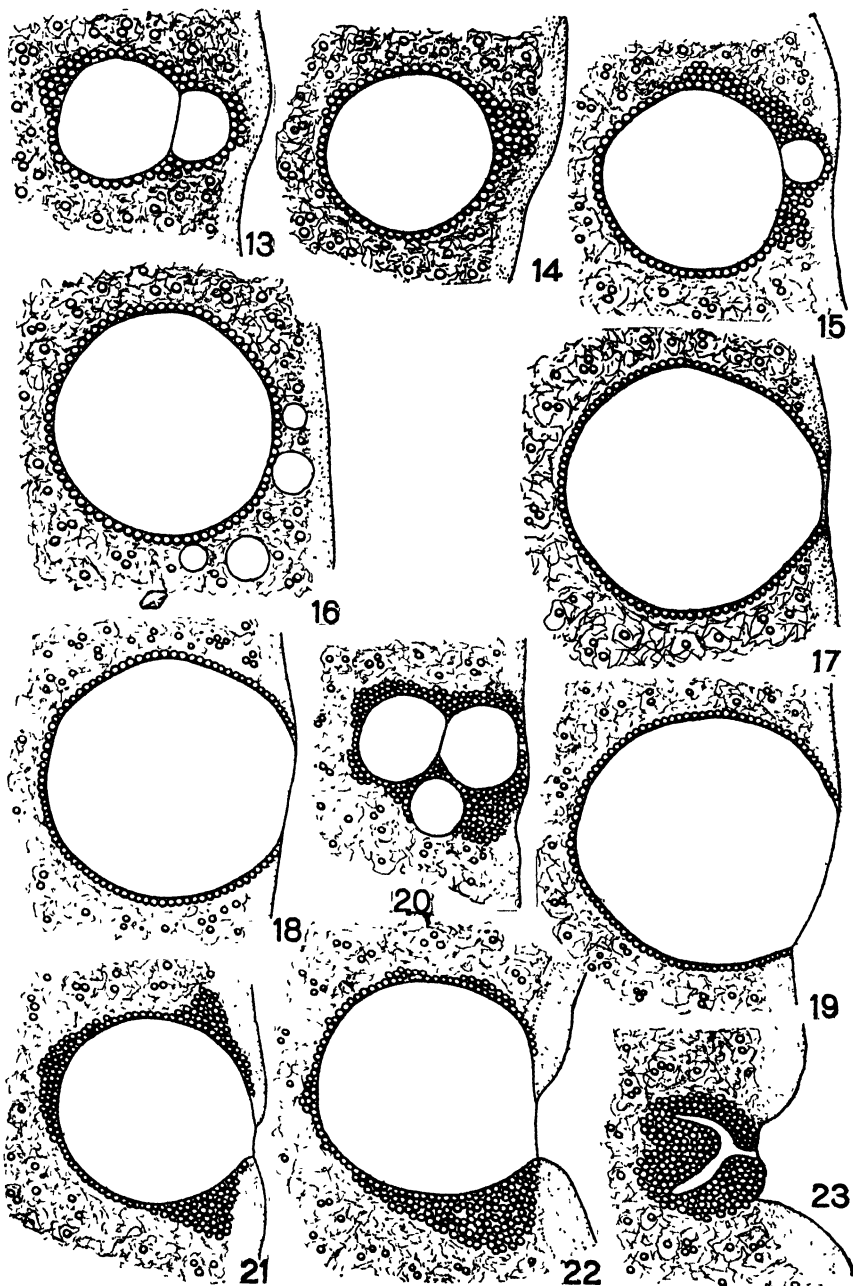
19 At 3:17½ p.m. Immediately before the rupture of the pellicle and the contraction of the vacuole.

20 At 3:23 p.m. During the five and a half minutes that intervened between the condition shown in the last figure and that shown in this figure, the vacuole contracted, leaving its mass of granules in contact with the pellicle. Several small vacuoles have appeared and have fused, till now we have one small and two large vacuoles.

21 At 3:27½ p.m. The single vacuole, the result of fusion, has not reached its customary full size, but is already in contact with the pellicle.

22 At 3:34 p.m. The vacuole is larger and is now ready to contract.

23 At 3:35 p.m. The vacuole is contracting, it having almost entirely disappeared. Even in this condition with the position of the pore so clearly shown by the shape of the vacuole, I failed to see the actual pore. I do not doubt it was of the full size indicated by the outlet tube, but one could not focus so accurately on the pore itself as to escape the impression of the pellicle above and below the pore. I have drawn this as it appeared, as if stretched across the mouth of the outlet tube. One cannot doubt however that there was here an actual pore.

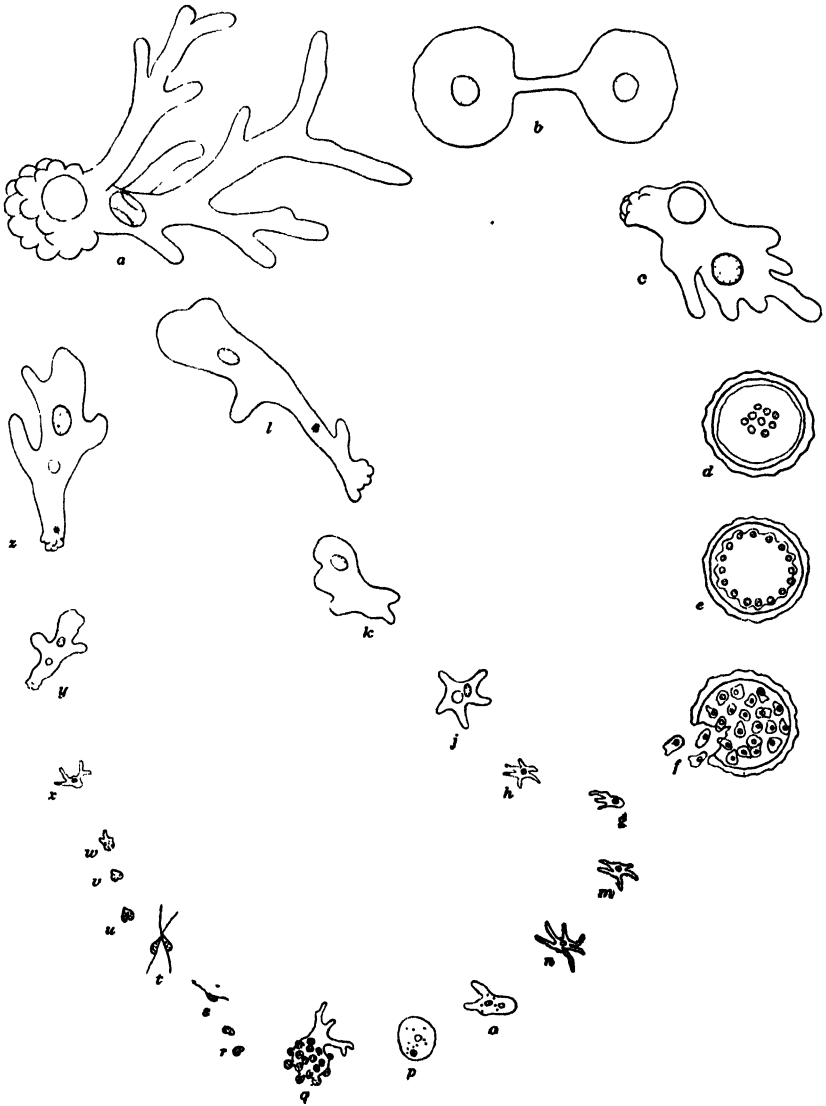


EXPLANATION OF FIGURES

24 *Amoeba currens*, n. sp.

25 Schema of hypothetical life-cycle of *Amoeba proteus*. a. Large "vegetative" individual (after Leidy). b. Large "vegetative" individual in fission (after Leidy). c. A somewhat smaller "vegetative" individual, a product of division. d-f. Stages in the formation of endoamoebozooids, as described by Scheel. These figures are too large in comparison with a-c, so also are figures m-w. g-l. Stages in the growth of these Endoamoebozooids into large "vegetative" individuals. m-q. Stages in the formation of gemmules. r. Amoeboid gemmules just freed, s. A similar gemmules which has become biflagellate. t-w. Copulation of flagellozooids to form an Amoeboid copula. x-z. Stages in the growth of such a copula into a large "vegetative" individual. The evidence that such individuals as that shown in figure n are derived from Scheel's endoamoebozooids is not satisfying. This schema is therefore hypothetical as to the interrelations of the several types of reproduction in the one life history.

24



25

EXPLANATION OF FIGURES

26-42 *Amoeba proteus*.

All drawings were made with camera lucida from living individuals.

26 Young *Amoeba* of the "radiosa" type. $\times 1500$ diameters.

27 Young *Amoeba* showing a few refractive plastids in the endosarc. $\times 1500$ diameters.

28 Young *Amoeba* showing nucleus, plastids and numerous vacuoles. $\times 1220$ diameters.

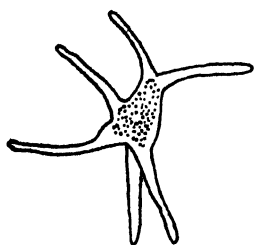
29 Young *Amoeba* with numerous plastids. $\times 1020$ diameters.

30 Small *Amoeba* with rather numerous plastids and a large number of food masses (conventionally shaded). $\times 1220$ diameters.

31-33 Stages in the formation of gemmules.

32 Some of the gemmules have already been freed and are lying near the *Amoeba*. In fig. 33 almost all the gemmules have been freed and have moved away. $\times 1500$ diameters.

34 An active amoeba with gemmules. $\times 1700$ diameters.



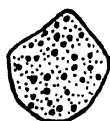
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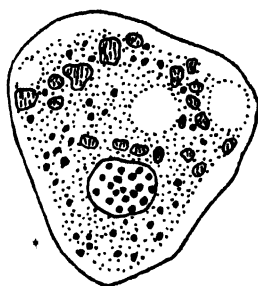
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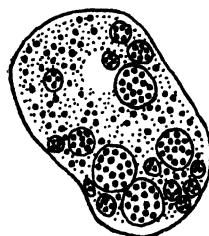
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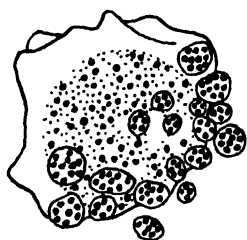
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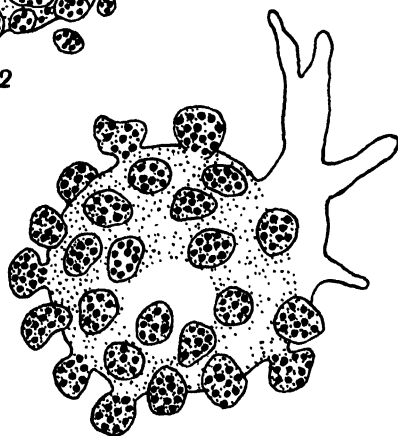
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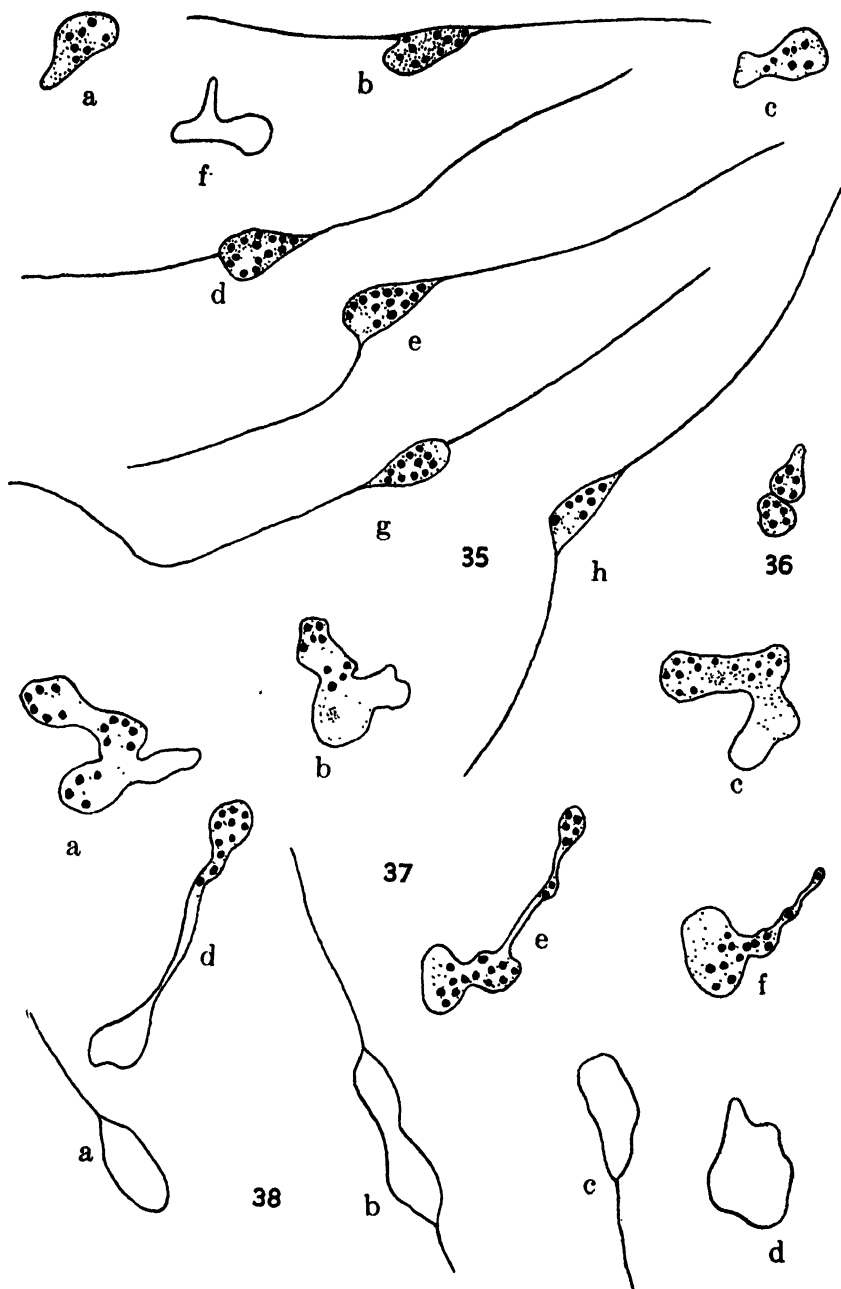
EXPLANATION OF FIGURES

35 **a-h** Amoeboid and flagellate spores which have arisen from gemmules. The same spore assumes "at will" either form. $\times 1590$ diameters.

36. An amoeboid-flagellospore in division, the two parts being about to separate. $\times 1590$ diameters.

37 **a-f** An amoebospore crawling on the cover glass and assuming grotesque shapes. In **b** and **c**, the nucleus is shown. $\times 1700$ diameters.

38 **a-d** Changes of form of one spore which is giving up the flagellate condition and becoming amoeboid. $\times 1590$ diameters.



EXPLANATION OF FIGURES

39 a-c Division of a spore which has pulled in its flagella. In c one of the daughter cells is beginning to grow flagella, one having appeared. $\times 1700$ diameters.

40 a-c Attempted copulation of flagellospores. They drew in their flagella after touching one another. In c one of the individuals has already reformed one of its flagella preparatory to swimming away. $\times 1700$ diameters.

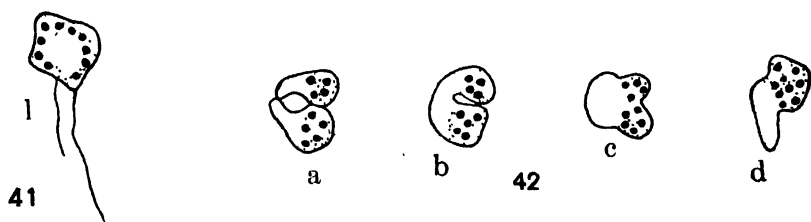
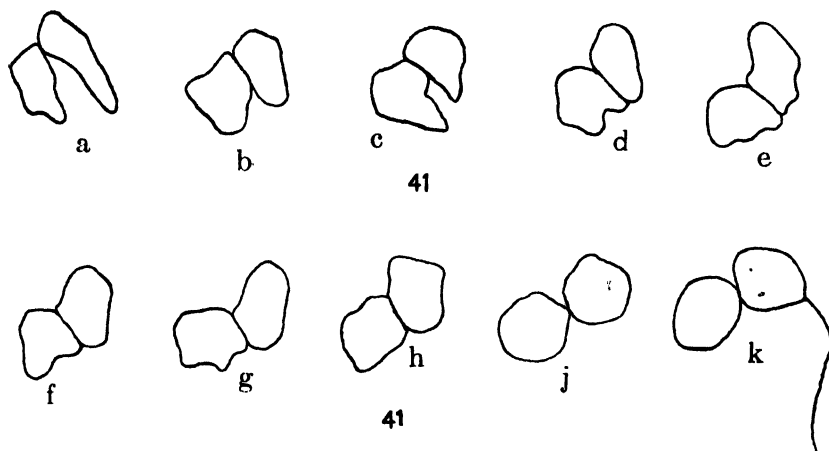
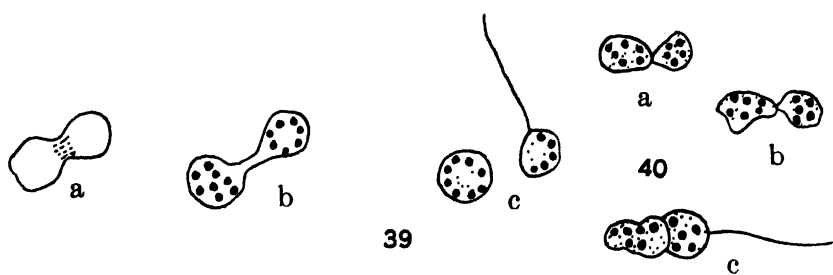
41 a-l A prolonged but unsuccessful attempt at copulation. Time, thirty minutes. Observe the change of form of the two individuals. In k, one individual has reformed one flagellum. Soon it formed a second and swam away. In l, the remaining individual has reformed its flagella (one of these is apparently bent back under the body so as to obscure the fact that the two flagella protrude from opposite ends of the body). $\times 1700$ diameters.

42 a-f Successful copulation of two flagellospores. They drew in their flagella promptly upon touching. The flagellate condition was not drawn because the outcome was not foreseen. $\times 1590$ diameters.

43-44 *Amoeba* sp.

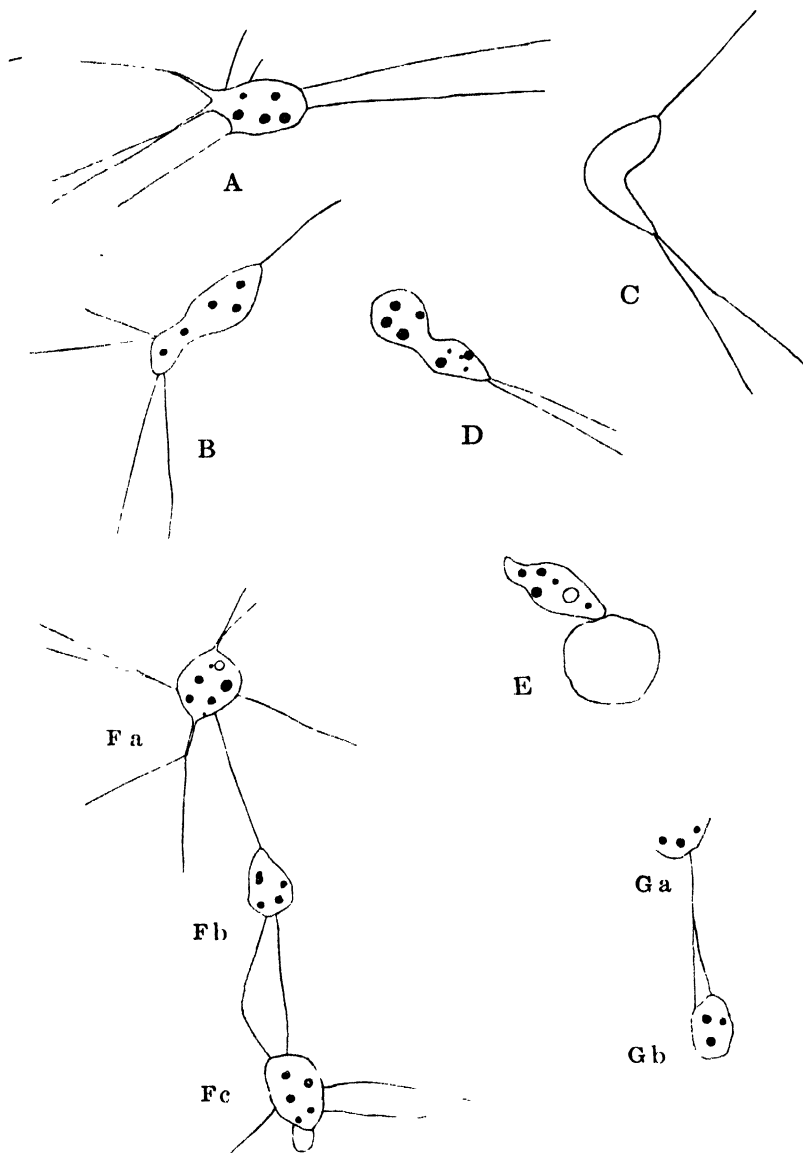
43 A gemmulating *Amoeba* of the smaller species. $\times 1700$ diameters.

44 Gemmules freed from such an *Amoeba* as that shown in fig. 43. $\times 1700$ diameters.



EXPLANATION OF FIGURES

45 A swarm spore of an undetermined species of *Amoeba* from Wurzburg Bavaria, drawn at brief intervals. Drawings *A* and *E* were made at intervals of one-half minute. *F* was drawn three minutes later, and *G* still a half minute later. *Fa*, *Fb* and *Fc* are three united individuals. *Ga* and *Gb* are the same individuals as *Fa* and *Fb*, respectively. *E* is in contact with a grain of debris of no significance. $\times 3030$ diameters.



THE EMBRYOLOGY OF STOMOTOCA APICATA¹

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THIRTY-TWO FIGURES

INTRODUCTION

The material for this research was secured, and the observations on the living forms were made, during the summers of 1903 and 1904 while I was occupying a table at the United States Bureau of Fisheries' Laboratory at Beaufort, North Carolina. *Stomatoca* is not very abundant in the harbor at Beaufort. I found it as early as the middle of June. It is most plentiful during July and early in August. A few specimens may also be taken until early in September. The eggs were obtained from medusae captured between July 10 and August 5. The adult animals could not be secured in large numbers; and, owing to the fact that each female lays only a few eggs, the material for embryological study was limited. Therefore the greater part of the work, the results of which are embodied in this paper, was done with living material. All the drawings with the exception of those of sections were made from camera sketches of the living forms. Blastulae and planulae ranging in age from five to twenty-seven hours were preserved and sectioned for the study of the various stages in the formation of the entoderm and the other features of development which make their appearance during this period.

¹ I wish to acknowledge my obligations to the Honorable George M. Bowers, Commissioner of Fisheries for the privileges afforded me at the Fisheries' Laboratory; and also to thank Dr. Caswell Grave, Director of the Laboratory, for help and suggestions. The work was finished at the Biological Laboratory of the Johns Hopkins University. Much interest was shown and kind suggestions were offered during my work by the late Professor W. K. Brooks.

DEHISCENCE

The eggs are discharged at about five o'clock in the morning. The ectodermal epithelium of the ovaries becomes ruptured, in fact broken down; and by the movements due to the muscular contractions of the manubrium the eggs are set free into the cavity of the sub-umbrella. Then by the rhythmic contractions of the bell they are forced out of the bell cavity into the water outside. While the eggs are being laid the medusa remains at one spot, unless disturbed, and keeps up a continuous and rhythmic contraction and expansion of the bell and proboscis. Thus as the eggs are liberated, one, two, or three at a time, they are almost immediately passed out with the ejection of the water from the bell cavity. The process of dehiscence lasts for a few minutes during which the medusa remains at the bottom of the aquarium. All the mature eggs are discharged without intermission in the process, unless the medusa is disturbed. In that case it frequently swims to another part of the aquarium and in a short time commences to discharge the eggs again. The eggs in the ovaries of *Stomotoca apicata* are usually all deposited at one time. Occasionally a few immature ones are left in the ovaries after the process of dehiscence. Whether these mature and are laid at a later time, or whether they are reabsorbed I am unable to decide.

As stated above, the eggs are laid at about five o'clock a.m. On several occasions I observed the process of dehiscence and found that the time was always practically the same. Some medusae were watched all night, July 14. At five o'clock in the morning they began to lay their eggs. They all began at about the same time and all the eggs were discharged within fifteen or twenty minutes. The time when the medusae are captured and put into the aquarium does not seem to have any influence on the period of dehiscence. I have taken them in the tow at nearly all hours of day and night, and never had them to deposit their eggs except at five o'clock in the morning.

THE EGG

The egg of *Stomotoca apicata* is spherical and measures .14 of a millimeter in diameter. It is devoid of a membrane and the cytoplasm is rather dense and only semi-transparent; however it is not as dense as the egg of *Stomotoca rugosa*, which is extremely opaque and of a chalky white color, and also slightly larger. The color of the egg of *Stomotoca apicata* is a bluish-white.

A point of interest may be mentioned in this connection. On one occasion, when I had taken a number of *Stomotoca* in the tow at night, they were picked out and put into a dish of clean sea-water with the intention of allowing them to lay and using the eggs for study the next morning. It happened that both species of *Stomotoca* that are found at Beaufort were represented. There were mature females of both species that deposited their eggs the next morning at the regular period; *Stomotoca rugosa* has the same time of dehiscence as *Stomotoca apicata*. Only the eggs of the latter species developed; there being no males of *Stomotoca rugosa*. The next day when the two species were in the same dish, and both discharged their eggs, only the eggs of *Stomotoca rugosa* segmented and developed. In this case there were no mature males of *Stomotoca apicata*. These facts aroused my interest and on several later occasions I placed the two species together with the intention of getting them to interbreed, but did not succeed and therefore I am led to the conclusion that they will not cross even though they are species of the same genus. To my knowledge no other experiments have been made in attempting to cross different species of this group of animals, and I did not have the opportunity to try with any other species than the above named after my attention had been called to the fact that they did not cross when accidentally placed in a dish together.

POLAR BODIES

Soon after the egg is deposited the first polar body is given off. A few minutes later the second polar body is formed. They remain near the egg for some time, frequently until after the sec-

ond or third segmentation. The polar bodies are not held by a membrane, as the egg is devoid of such a structure; neither are there any protoplasmic connections visible with a magnification of 212 diameters. Yet for a time they seem to be held near the egg by some means of attraction. The first polar body may segment once or twice. Usually about the time of the second cleavage the polar bodies either disintegrate or pass out into the water and are lost.

FERTILIZATION

Very little concerning fertilization could be made out on account of the character of the egg. The ova and spermatozoa are discharged into the water and there fertilization takes place. It is impossible to follow the nuclear changes which take place during maturation, or the union of the male and female pronuclei in the living egg, because of the density of the cytoplasm, and because material could not be secured in sufficient abundance in the various phases for the preservation of the different stages for sections. There is no visible fertilization membrane given off after the penetration of the spermatozoa.

CLEAVAGE

Cleavage is total, equal and nearly regular, especially in the early stages. The divisions occur at short intervals, and the blastomeres soon move away from the center of the egg, thus forming a gradually enlarging segmentation cavity. The cells continue to divide and arrange themselves into a single layer around the blastocoele to form a true blastula. The egg is not divided into an animal and a vegetative pole as the deuterooplasm and protoplasm are distributed evenly in all parts. But, as is customary, and for convenience of description I will call the part of the ovum from which the polar bodies are given off the upper pole, and the part of the egg opposite the lower pole.

The first cleavage occurs a short time after the polar bodies are ejected. The plane of division is vertical; the segmentation furrow begins at the upper pole and gradually deepens until the

egg is cut into two equal parts. The egg, viewed from above, at first shows a nearly circular depression which very soon spreads laterally and begins to grow down. This first furrow is wide and leaves the blastomeres separated from each other as it progresses downward, as is seen by looking at the egg from the side (figs. 4 and 5). This furrow remains open until the egg is almost separated into two parts; the blastomeres being connected simply by a narrow protoplasmic film at the lower pole. Protoplasmic currents can frequently be seen in this connecting thread. Bunting (1893) describes and figures in *Hydractinia* a protoplasmic thread in the two-celled stage in which she also notes protoplasmic movements. The connecting film in *Stomotoeca apicata* is not as clear and definite in outline as she shows it in her figure of *Hydractinia*. The two cells gradually come into close proximity and in a short time the connection of protoplasm at the lower pole is broken and the complete two-celled stage is found (fig. 6).

The second plane of division is also meridional and at right angles to the first. This cleavage takes place about fifteen minutes after the first. These second segmentation furrows start at the center and move out toward the periphery. During their progress outward there are to be seen globular or oval spaces at their outer extremities. These spaces are large enough to cause openings that extend through the egg as shown in fig. 7. During this cleavage there is a shifting or rotation of the blastomeres from right to left. The second segmentation furrows usually start opposite each other at a point in the center of the first cleavage furrow, and then are carried apart by rotation; or the rotation may have started before the second segmentation began, in that case the second cleavage planes are some distance apart as soon as they make their appearance. Fig. 7 shows an egg in the process of division in which rotation has taken place. During the progress of the second segmentation, the egg has frequently a flattened appearance as seen in the figure just mentioned.

In this stage protoplasmic films or bridges, also, frequently exist for a time after the segmentation is practically complete. They are finally absorbed by the blastomeres which then round up and form the completed four-celled stage as shown in fig. 8.

The third cleavage plane is equatorial and divides the egg into eight equal blastomeres; four of which are situated at the upper pole and four at the lower pole of the egg, as seen in fig. 9. This is the condition when segmentation is regular, and may be described as two four-celled stages of half size superimposed one upon the other and then the upper set rotated to the left. While the formation of the eight-celled stage was always nearly the same in the eggs that I followed, after the division was completed, the blastomeres did not always retain the same relative positions. Sometimes there occurred a separation of the cells at one side of the equatorial furrow and the blastomeres rolled apart in such a manner as to form a curved sheet. In others this separation and unrolling of the blastomeres was less definite and the final arrangement was such as is shown in fig. 10

The irregularity in the relative position of the blastomeres begin with the eight-celled stage and is more or less characteristic of all later stages up to the formation of the blastula. While there is diversity of arrangement of the blastomeres, nevertheless I am led to believe that the division of the individual cells is regular and takes place just as though the blastomeres always held the same relative position.

The fourth segmentation follows after a short period of time. Fig. 11 shows a sixteen-celled stage which is nearly regular, but the cleavage cavity has already been formed within the mass of blastomeres and they are thus pushed away from the center of the egg. In this stage the cell lineage can be traced even in the forms that are somewhat irregular. But in the older stages the arrangement of the cells is more irregular and, owing to the opacity of the egg, it is difficult to follow with accuracy the descent of the cells. Fig. 12 shows a later stage in which the arrangement of the cells is more irregular than is common in eggs of the same age.

As stated before, the divisions follow each other at short intervals. Within two hours after the eggs were laid they had undergone the processes of maturation and fertilization, and had passed beyond the sixty-four-celled stage. The cells continue to divide with the same rapidity, while within them the cleavage cavity is also gradually enlarging. Fig. 13 shows a stage in which the

cells are more or less definitely placed around the segmentation cavity. The blastomeres finally become very numerous and small, and arrange themselves around the blastocoele in a single-celled layer forming a true blastula.

BLASTULA

The blastula is oval in shape, and is but slightly larger than the unsegmented egg. The average size of several blastulae that were measured was .19 mm. in length and .15 mm. in their largest transverse diameter. The egg before cleavage measured, as stated before, .14 mm. in diameter. The blastomeres in the blastula stage have become very numerous and small, and arranged in a single layer of epithelial cells. When the larva is about eight or ten hours old, these peripheral cells develop cilia; probably each cell has one cilium. With the development of the cilia, movement commences. At first the motion is very slight, but as the cilia become more numerous, the blastula is enabled by the ciliary movements to leave the bottom of the aquarium upon which it lay and to swim about in the water with a spiral or cork-screw motion that is characteristic of hydroid blastulae and planulae. The large end of the blastula is directed forward and therefore may be called the anterior end. Whether the anterior part of the larva corresponds to the upper or to the lower pole of the egg was impossible to determine. It is reasonable, however, to infer that there may be no fixed polarity in the larva of Hydromedusae, for it is well known that normal embryos of small size will develop from fragments of eggs.

PLANULA

The blastula gradually elongates and becomes narrower forming a larva which is usually about three times as long as broad and known as a planula. From measurements taken of living planulae the average size is about .25 mm. in length and .08 mm. in the short diameter. These measurements are not constant, the larva becoming somewhat longer at an older age. The anterior end

remains slightly larger than the posterior, but the difference is not as great as in the blastula. During the blastula stage the larva swam near the bottom of the dish; when it attains the planula stage it rises and swims at or near the surface of the water for a shorter or longer time. This phenomenon occurs about twenty-four hours after the egg is fertilized. After several hours the planula gradually settles toward the bottom again and finally the spiral movements cease, due to the loss of the cilia. For a time of varying length after the spiral motion stops, the planula glides along the bottom of the aquarium. About forty-eight hours after the eggs are laid the larva reaches the stage of development in which attachment takes place. In preparation for attachment the planula settles to the bottom, loses its cilia and ceases its movements.

FORMATION OF THE ECTODERM

The formation of the ectoderm in *Stomatoca apicata* is simple in comparison with that of those species in which the segmentation of the egg is unequal, giving rise to macromeres and micromeres; and in which the ectoderm is formed by a rapid increase of the micromeres and overgrowing of the macromeres by the process of epibole. In *Stomatoca*, on the other hand, the cleavage is equal and at the completion of segmentation the blastomeres have divided into cells of uniform size and are situated in a single epithelial layer around the periphery of the blastula (figs. 16 and 17 show sections of blastulae five and eight and one-half hours old respectively). Thus, from their position, all the cells which directly result from the segmentation of the egg may properly be regarded as forming ectoderm; and indeed might already at this stage of development be designated as such, were it proper to use the term ectoderm before the appearance of the inner germ-layer. The cells of the blastosphere are columnar in shape and, at first, all are comparatively of the same height; but finally those cells at the posterior end become somewhat taller than the rest. This is the region where the entoderm will be budded off.

FORMATION OF THE ENTODERM

In *Stomatoca* the formation of the entoderm takes place by unipolar ingression, or the "hypotrope" method. The latter term was used by Metschnikoff in contradistinction to multipolar migration. In the multipolar formation of the entoderm he distinguished four different modes, namely: 1. A primary delamination which takes place by a transverse division of the blastoderm cells, and occurs in the Geryonidae and *Eudendrium*. 2. A multipolar ingression which takes place on all sides (*Aeginopsis*). 3. A secondary delamination which occurs where a morula structure exists, as in *Aglaura*, *Rhopalonema* and in most of the hydroid polyps. 4. A mixed delamination in which the entodermal cells originate in part through transverse division or ingression; and also through subsequent differentiation as a secondary delamination. This last mode of the formation of the entoderm, according to Metschnikoff, occurs in *Polyxenias*; and is the transitional method between multipolar migration and epibole. In the unipolar ingression, or "hypotrope" process, the formation of the ectoderm is confined to a comparatively small area at the posterior end of the blastula. This is the method that is followed in the species under consideration.

About the time the blastula becomes ciliated and begins to swim, usually eight to ten hours after fertilization, the cells at the posterior end of the larva become somewhat taller than those in the other regions, and from these cells, relatively few in number, the entoderm arises. The formation of the entoderm in *Stomatoca* is, in a general way, similar to that described and figured by Metschnikoff in his "Embryologische Studien an Medusen" for *Clytia flavidula*, *Clytia viridicans* and *Octorchis Gegenbauri*. The entoderm cells are given off from the lower end of the blastula and are pushed into the blastocoele. At first a single cell may be budded off. Gradually more cells are given off, and those first set free divide; so that by the continuation of this process for an indefinite time, the blastocoele becomes filled solidly from the posterior to the anterior end. Fig. 18, 19 and 20 are from sections of blastulae in which the formation of the entoderm is in different

stages of progress; and in fig. 21 the entodermal tissue has filled the entire cavity.

According to Metschnikoff, in his description of unipolar ingression or "hypotrope," the entodermal tissue arises as a rule by bodily migration of ectodermal cells into the blastocoele, and not by a transverse division of the ectodermal cells,—the inner parts going to form entoderm and the outer parts remaining as ectodermal cells. In fig. 20, plate 2 Metschnikoff shows a cell in the process of transverse division; and in fig. 21 of the same plate two cells are so situated that one can easily infer that they may have arisen by transverse division of a single ectodermal cell. These figures are of *Clytia* and in his description of the same species he mentions the cell in fig. 20 as the only one that he found in which transverse division occurred. This he seems to regard as an exception, and claims that as a rule the ectodermal cells increase by longitudinal division and migrate into the interior.

My material for studying the formation of the entoderm in *Stomatoca* was scarce and it is not impossible to have misinterpreted the phenomena. However, I am inclined to think that the entodermal cells arise by a transverse division of the ectodermal cells, as Metschnikoff shows in the exceptional case of *Clytia viridicans*. Fig. 18 is drawn from the only section I was able to secure from preserved material showing the beginning of the formation of the entoderm; and that section was cut slightly oblique, causing some doubt. A section of a little older stage and drawn with a higher magnification is shown in fig. 19. Here there are three cells that appear to have just divided by transverse division. Another reason which causes me to think that the entodermal cells arise by transverse division of the original ectoderm cells is the fact that the ectodermal cells in this region are practically as wide as those in the other parts of the blastula. This would not be the case if the longitudinal division occurred; for cell division is necessarily more rapid in the region where the entoderm is given off, and consequently the cells would be narrower. Unfortunately, because of scarcity of material, the exact cellular details of the formation of the entoderm will have to be left for future study.

The migration of the entoderm continues for some hours, and finally the blastocoele becomes solidly filled with this newly developed tissue. At first the cells are crowded together without any definite arrangement except that due to pressure. Then those cells that are situated next to the ectodermal layer change shape, becoming columnar and assuming the appearance of a more or less distinct layer. Such an arrangement is shown in fig. 22. Later a separation of the cells takes place in the center of the entodermal mass. This is the first beginning of the coelenteric cavity, which gradually increases in size; and finally the entodermal cells become arranged in a single layer around this cavity.

DIFFERENTIATION OF THE ECTODERMAL CELLS

When the larva is about twenty-four hours old and about the same time that the entodermal tissue begins to arrange itself into the definite inner germ layer, a differentiation begins in the ectodermal tissue. The interstitial cells now make their appearance here and there by crowding in between the bases of the columnar ectodermal cells. These latter cells which heretofore were straight, cylindrical structures with their sides parallel to each other, now become more irregular; some assume conical forms, others spindle shapes according to the pressure of the neighboring cells. Also, about this time, or a little later, small oval refractive bodies make their appearance usually in the interstitial cells, occasionally in the entodermal cells also. These small ovoid structures gradually push their way toward the exterior, and finally come to be situated in or between the ectodermal cells at the surface. They are developed into nematocysts.

ATTACHMENT

When the larva is about forty-eight to fifty hours old it settles to the bottom, loses its cilia and thus its power of movement is lost. It is now ready to become attached. The method of attachment in *Stomatoca* differs from that usually described and regarded as typical for the hydroid larva; in which case they settle

down on the broad anterior end, from which the hydrorhiza are given off, while the opposite end forms the hydranth and develops the mouth and tentacles. The planula of *Stomotoca* instead of settling down on the anterior end, becomes attached by the whole length of the larva. That is, the planula does not become transformed into a hydranth but forms the root; and the first hydranth is given off from the root as a bud. The planula changes its shape about the time it is ready for attachment. The enlarged anterior end is reduced in size and the larva becomes spindle shaped. Then usually about the time the bud which will form the hydranth appears, the primary root branches, giving off one or two secondary roots; so that when the hydranth is developed it may have two, three or four hydrorhiza as shown in figs. 27 to 32. The settling down and attachment of the planula of *Stomotoca apicata* is very much like that which takes place in *Turritopsis nutricula*, the development of which was described in a recent paper.

Professor W. K. Brooks in his work on "The Life-History of *Eutima*" (1884) has shown that the planulae of *Eutima*, *Turritopsis* and *Hydractinia* form roots and that the hydranths arise as buds from the roots.

DEVELOPMENT OF THE HYDRANTH

After the larva has become attached it very soon develops a bud which is the beginning of the hydranth. A circle of small projections make their appearance very early around the distal end of the hydranth bud; these are the rudiments of the tentacles and are usually five in number. Occasionally a hydranth bud has six tentacular projections and thus gives rise to six primary tentacles. The mouth is now developed, as a slit breaking through the two germ layers at the apex of the young hydranth in the center of the whirl of tentacular buds. About a day later more tentacles appear. These secondary tentacles alternate with the primary one. The secondary tentacular buds do not all appear simultaneously; but are usually added one or two at a time until the second cycle of tentacles is completed and the hydranth has ten tentacles in all. Thus we may have young hydranths with six, seven, eight,

nine or ten tentacles according to the stage of development. Ten seems to be the number of tentacles in the fully developed hydroid, polyp. The oldest polyps that I reared, five days old, had this number; and Professor Brooks describes the hydroid, which he found on the lower surface of the shell of the living *Limulus*, and which had medusa buds developed, as having only ten tentacles. The hydranths that I reared in the Laboratory corresponded with those found by Professor Brooks and I have no doubt that they were the same species. The primary and secondary tentacles arise from the same level so that they may be said to constitute one whorl. The five primary tentacles, however, are longer and project forward; while the secondary ones are shorter and extend backward. The tentacles are well armed with thread cells which are arranged around the tentacles in clusters at short distances from each other, from one end of the tentacle to the other. These groups of thread cells become closer together as the distal end of the tentacle is approached.

A thin, delicate perisarc is secreted early in the development of the hydranth. It adheres closely to the root and stem. It does not extend the entire length of the stem; but stops a little distance below the circle of tentacles. In fig. 31 a polyp is shown in which the coenosarc has retracted for some distance in one of the hydrorhiza and left the delicate tube of perisarc empty.

SUMMARY

1. The eggs are laid at a regular time, about five o'clock in the morning. They are set free by the breaking down of the epithelial layer of the ovaries.

2. The egg is spherical and measures .14 mm. in diameter. It is destitute of a membrane when laid, and none is subsequently developed. The cytoplasm is dense and opaque.

3. Maturation takes place after the eggs are laid; and fertilization takes place very soon. Details of fertilization could not be made out because of opacity of the egg.

4. Cleavage is total, equal and nearly regular, especially in the early stages. Protoplasmic threads or bridges connecting

the different blastomeres during the early cleavages, are frequently encountered. The segmenting cells arrange themselves around a continually enlarging cleavage cavity.

5. At the completion of segmentation a true blastula is formed, which develops cilia and swims with a spiral motion. The oval blastula elongates and is transformed into a planula.

6. The ectoderm arises directly from the segmentation cells which are arranged in a peripheral layer around the blastocoele.

7. The formation of the entoderm is by unipolar ingression. The cells at the posterior end of the blastula bud off the primitive entodermal tissue which migrates into the blastocoele; and later is arranged into the inner germ layer.

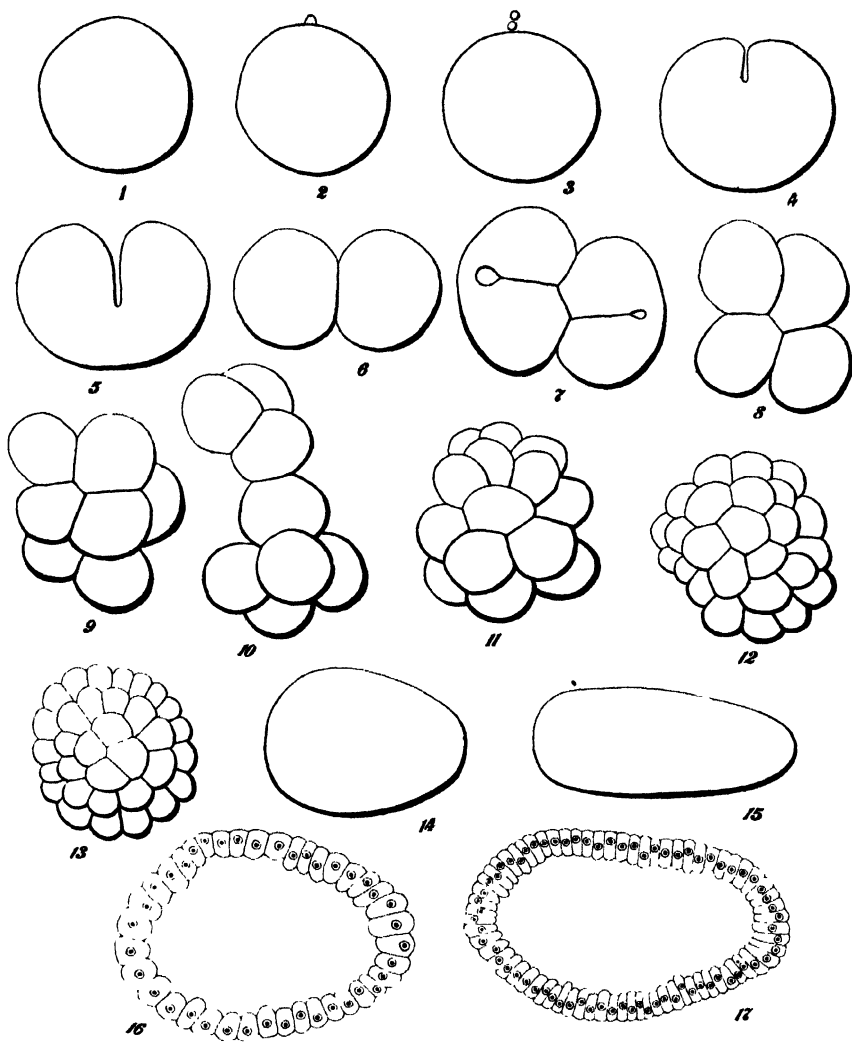
8. Nematocysts arise chiefly in the interstitial cells, sometimes in the entoderm, and migrate to the surface.

9. The larva becomes attached by its side and is transformed into the hydrorhiza. The root frequently branches soon after attachment.

10. The hydranth develops from a bud, which is given off from about the center of the hydrorhiza.

11. The tentacles appear early as small projections at the distal part of the hydranth bud.

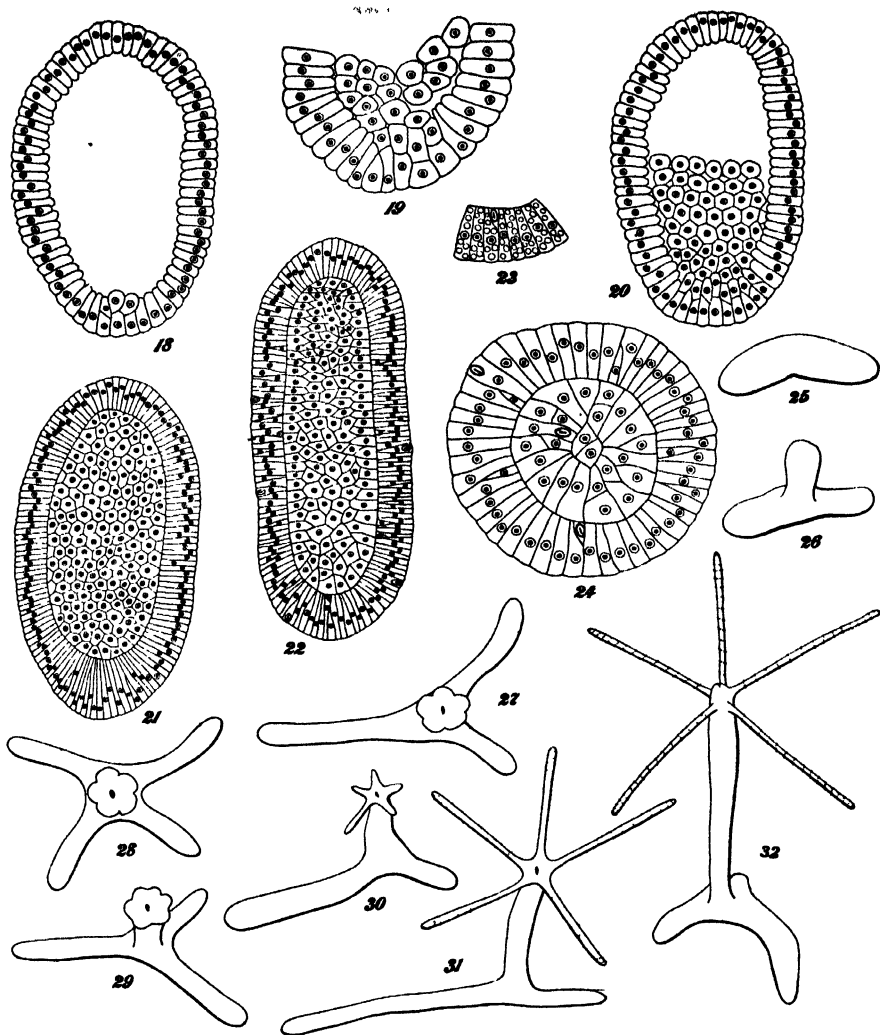
12. A thin, delicate perisarc is secreted around the hydrorhiza and stem up to near the tentacles.



Polar bodies, segmentation of the egg and formation of the blastula and planula.

- 1 Egg soon after it was laid.
- 2-3 Formation of polar bodies.
- 4-5 Fertilized egg with first segmentation furrow beginning at the upper pole.
- 6 The two-celled stage.

- 7 The four-celled stage in formation.
- 8 The four-celled stage complete.
- 9 The eight-celled stage.
- 10 Another eight-celled stage.
- 11-12 Later stages of segmentation.
- 13 A young blastula.
- 14 An older blastula, in outline.
- 15 A planula.
- 16-17 Sectional views of blastulae.



Formation of the endoderm, attachment of the larva and development of the hydranth.

- 18 Section of blastula with endodermal cells just beginning to bud off from posterior end.
- 19 Section of posterior end of blastula with a few endodermal cells given off.
- 20 Blastula with more endodermal cells migrating into blastocoele.
- 21 Blastula with blastocoele full of endodermal cells.

- 22 Planula with endodermal cells becoming re-arranged.
- 23 A few ectodermal cells with higher magnification, showing an interstitial cell and nematocyst.
- 24 Cross section of planula a little older than one shown in longitudinal section in figure 22.
- 25 Larva at the time it settles down.
- 26-32 Attached larva with hydranth bud in various stages of development.

THE LATERAL LINE SYSTEM OF CHIMÆRA COLLIEI

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EIGHTEEN FIGURES

INTRODUCTION

The interesting systematic position of the Chimæroid fishes, and the somewhat confused classification of the members of the group, would seem to justify a description of the lateral line system of this particular species even were the system not of sufficient interest in itself to make such a piece of research worth while.

Dean's recent monograph ('06) makes it unnecessary to discuss the appearance and habits of this species. His beautiful figure of the adult animals shows quite accurately the surface appearance of the canals, as seen in a lateral view, but he gives no description of the system.

According to Dean's classification there are four genera and something more than twenty-five species of Chimæroids.

Garman says there is no greater divergence in the most dissimilar forms of sharks than there is in the lateral line system of two genera of Chimæroids, *Callorhynchus* and *Chimæra*.

The Chimæroids are widely distributed over nearly the entire world. The species under discussion is found along the Pacific coast of the United States, and nearly all of the material upon which the present work was done was collected at Pacific Grove, California.

I am indebted to the Directors of the Hopkins Marine Laboratory for the privilege of working at their station, and to Professor Ritter for his courtesies during a most pleasant and profitable visit at the San Diego Marine Biological Station, though no fresh Chimæroid material could be obtained at the latter place.

Owing, probably, to the fact that the fish could not be obtained until several hours after death, the material that was preserved for histological study was not well fixed, so that the finer histological details could not be made out.

There is, of course, a considerable literature upon the lateral line system of sense organs, and several workers have published researches upon this system in the Chimæroids. Nothing, however, so far as I am aware, has been published upon the lateral line in the species under discussion.

THE DISTRIBUTION OF THE CANALS

It will be well to begin the discussion with a description of the distribution of the canals, since it is the superficial appearance that is, naturally, first noticed and used in taxonomy.

The nomenclature adopted by Garman for the canals seems to be the simplest and most reasonable, and it will be used in this paper. The names used by Garman are, he says, with few exceptions, those adopted by Agassiz.

Except in one or two cases, on the ventral side of the head, the course of the canals is remarkably clear and definite, and in the numerous specimens examined but few variations in their position could be noticed.

The system as a whole may be divided into the cephalic and the corporal canals (fig. 1); the former form a complicated system of lines extending over practically the entire head, while the latter consist of the single lateral canal on each side, that is such a universal characteristic of the group Pisces. In some elasmobranchs the corporal system includes also a pleural canal, extending out upon the pectoral fin; this canal is not present in *Chimæra*.

The lateral canal (fig. 1, *l*, *l'*) extends from its union with the occipital and orbital, of the cephalic system, a short distance back of the eye, to the tip of the slender, tapering tail. A short distance from its anterior end, under the base of the spine of the anterior dorsal fin, it has a slight, but constant, upward bend. From this bend the canal extends caudad in a direction approximately parallel with the dorsal body line, and, in a full-grown fish, at an

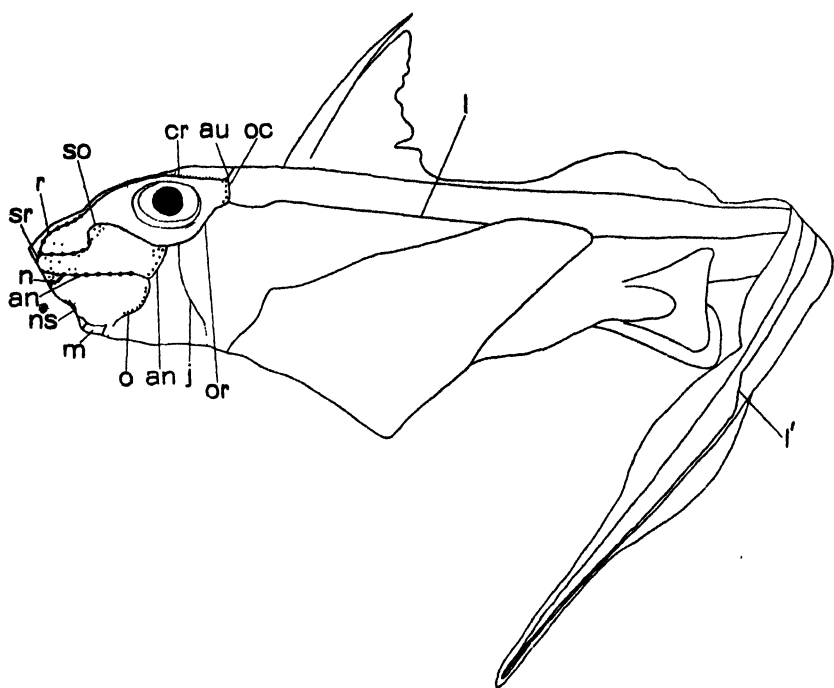


FIG. 1 A lateral view of *Chimæra colliei*, showing the distribution of the canals of the lateral line system. For explanation of lettering in all figures see p. 370.

average distance of two or three centimeters from it. At the region between the posterior dorsal and the caudal fins, where the dorso-ventral thickness of the fish is slight, the canal is somewhat nearer the dorsal than the ventral body line. Just back of this point, however, the line drops rather suddenly to the lower edge of the body and extends to the end of the tail close to the union of the caudal fin with the body (fig. 1, *l'*).

According to Garman the course of the lateral canal in *C. monstrosa* ('88) is practically the same as in *C. colliei*, while in *Rhinochimæra pacifica* ('04) it is essentially the same, except for the absence of the upward bend near its anterior end. In *Callorhynchus antarcticus* Garman ('88) figures the lateral line about as it is in the ordinary sharks, without either the anterior upward bend or the posterior downward one. Garman neither

describes nor figures the lateral canal in the fourth genus of the group, *Harriotta*, and the figures of Dean ('06) and of Jordan and Evermann ('00) are so small that the exact course of the canal is difficult to determine. Its anterior bend seems, however, to be about the same as in *C. coliei*, while its posterior portion, along the ventral edge of the body seems to be due rather to an upward tendency in the ventral outline of the body than to a downward bend in the lateral line itself.

The cephalic system of canals is very complicated but, as might be expected, is quite uniform in arrangement in individuals of the same species, though some variations are noticed.

None of the cranial canals has the appearance of being a direct continuation of the lateral canal, but the orbital (fig. 1, *or*) most nearly approaches this condition. It begins one to two centimeters back of the orbit, where it is continuous with the lateral (*l*) and the occipital (*oc*). Extending cephalad and ventrad it gives off, or unites with, two canals, the angular (*an*) and the jugular (*j*), at a point about one centimeter below the eye, and continues cephalad as the suborbital (*so*). The course of the orbital is about the same in all the *Chimæroids*.

The suborbital (figs. 1, 2, and 4, *so*) is a direct continuation of the orbital. It passes cephalad and dorsad to a point about half-way between the orbit and the tip of the snout; there it makes a wide bend downward and continues cephalad to unite with the rostral (*r*) and subrostral (*sr*). In the region anterior to the bend just mentioned the subrostral canal has a series of five or six enlargements that will be described later. Along the concave side of the bend and in the angle between the suborbital and the angular is seen a number of small, round, glandular openings. In *C. monstrosa* and in *Callorhynchus* this canal follows about the same course as has just been described, except that, in the latter form, the bend is not so marked. In *Rhinochimæra* and *Harriotta* the bend is less marked and the anterior region is longer, because of the greater length of the snout in these two forms. The enlargements in this region of the canal are also wanting in these two species, so far as can be determined from the figures of Garman ('04), Dean ('95), and Jordan and Evermann ('00).

Extending in a dorso-median direction from its point of confluence with the lateral and orbital is a canal whose entire length is not more than one or two centimeters; it has been called the occipital (figs. 1, 2, *oc*). Along its anterior side is seen a number of the small glandular openings that were mentioned above. In one specimen was seen a small, Y-shaped branch (fig. 2, *y*), about one

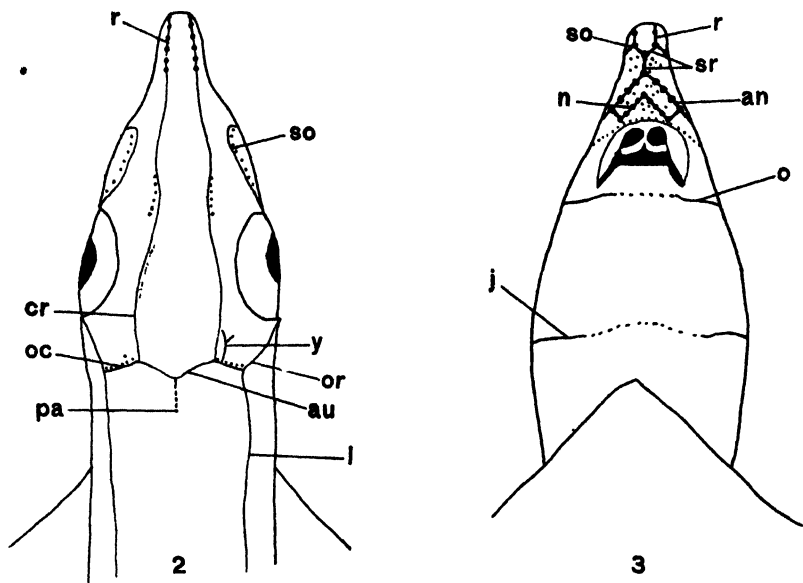


FIG. 2 A dorsal view of the head of *C. colliei*, showing the arrangement of the cephalic canals.

FIG. 3 A ventral view of the head of *C. colliei*, showing the distribution of the canals.

centimeter long, extending cephalad from the occipital canal of the right side. This was the only case of evident asymmetry in the arrangement of the canals that was seen. In *C. monstrosa* this canal has, as in the case of *C. colliei*, the appearance of being simply the continuation of the aural canal (*au*), while in *Rhinochimæra* and *Harriotta* it extends cephalad in such a direction as to seem continuous with the cranial canal (*cr*).

Extending across the top of the head, about half-way between the orbits and the dorsal spine, from the points of confluence of

the cranial and occipital of each side, is the aural canal (*au*), which may be considered as consisting of two lateral portions extending mediad and uniting at the mid-dorsal line. The point of union is caudad to the lateral ends of the canal, giving the shape of a wide V, when viewed from above (fig. 2, *au*). From the point of this V, Garman ('88) describes a short, median canal extending about half-way back to the base of the dorsal spine; he calls it the post-aural. This canal, since it was present in less than a third of the specimens of *C. collei* examined, has been shown in the dorsal view by a broken line (fig. 2, *pa*). Garman does not figure the post-aural in either *Callorhynchus* or *Harriotta*. In the specimen of *Harriotta* described by Garman the aurals do not meet in the mid-dorsal line, but overlap for about half their length; he does not say whether or not this is the normal condition, and other figures of the animal, at hand, do not make clear this point. The lack of fusion in this case would seem to justify the assumption that there are two lateral aurals rather than a single canal.

Extending cephalad, along the top of the head, from the point of confluence of the occipital and aural canals is the cranial canal (figs. 1, 2, *cr*). From their points of origin the two cranials diverge slightly, then converge somewhat, in the region of the orbits; then, after a second slight convergence, in front of the orbits, they converge gradually till they pass over the tip of the snout. On the medial side of each cranial canal, in the region of the orbits, is a considerable number of fine pores; and on the lateral side of each canal, just anterior to the orbital region, are half a dozen or more pores of larger size, like those mentioned in connection with the occipital and suborbital canals.

The anterior portion of the cranial canal, where it passes out over the snout, has been called the rostral (figs. 1, 2, *r*). There is nothing to indicate where the cranial should end and the rostral begin, unless we consider the rostral to be that part of the canal where the enlargements, like those noted in connection with the suborbital canal, are found. Passing over the end of the snout the rostral meets the suborbital and becomes the subrostral. The course of the cranial and rostral canals is strikingly alike in all the *Chimæroids*, as far as can be determined by the examination

of figures; but neither Garman nor Dean show any enlargements of the rostral canals of *Callorhynchus*, *Rhinochimæra*, or *Harriotta*.

Extending ventrad from the orbital canal, directly below the orbit, are two canals, the angular and the jugular. The jugular (figs. 1, 3, *j*), which arises about two or three centimeters posterior to the angular, as it passes ventrad curves, for a considerable part of its length, towards the pectoral fin; as it passes to the under-side of the fish, however, it comes to lie in a transverse direction. Near the mid-ventral line, where the two jugulars meet, they form a slight curve whose convex side is towards the head (fig. 3, *j*). For a considerable distance across the ventral side of the head the jugular is not a continuous canal, but is broken up into a series of short sections, like dots and dashes (fig. 3, *j*). In some specimens these dashes are very distinct, in others they can scarcely be traced from side to side. The course of the jugular canal, as well as can be judged from Dean's and Garman's figures and Garman's descriptions, seems to be about the same in all the Chimæroids that have been studied, except that in *Rhinochimæra* and *Harriotta*, as seen in Garman's figures, the dashes of the two sides do not quite meet each other in the mid-ventral line.

The angular canal (figs 1, 3, 4, *an*) arises, as has been said, from the orbital, a short distance anterior to the jugular. The position of either the angular or the jugular may be said to be the line of division between the orbital and the suborbital. For one or two centimeters the angular extends ventrad with a slight curve cephalad; then it makes a sudden turn cephalad and proceeds, in an almost horizontal direction, sloping slightly ventrad, towards the front of the snout where it unites with the suborbital. At the point where the angular turns suddenly towards the snout arises the oral canal, to be noted below. That part of the angular canal between the orbital and the oral is of the usual type, while the part between the oral and the subrostral has a series of wide spaces like those noted in connection with the rostral and suborbital. Along the anterior border of the vertical part of the angular are five or six large gland openings; and along the upper side of

the horizontal part of the canal, especially towards its anterior end, is a row of smaller openings. According to Garman ('88) the angular extends only to the point where the nasal is given off, anterior to which point it is known as the subrostral. As there is no change whatever in the character or direction of the angular at this point there seems to be no reason for changing its name, so that it will here be called the angular from the point of union with the orbital to the point of union with the median subrostral, on the antero-ventral side of the snout. In one of the two specimens of *C. monstrosa* studied by Garman the angular arose from

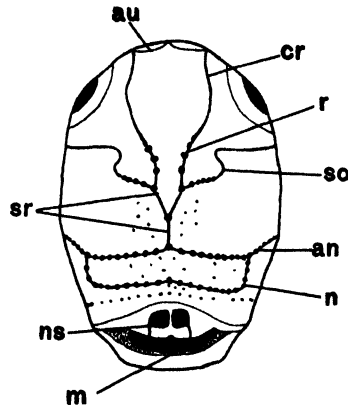


FIG. 4. An anterior view of the head of *C. collici*, showing the distribution of the canals.

the jugular, just after the latter came off from the orbital; in none of the numerous specimens of *C. collici* studied by the writer was this the case. Hubrecht ('76) figures the angular as arising from the orbital as in the present form. The exact course of the angular canal in *Rhinochimæra* and *Harriotta* could not be determined from Garman's description, nor from his figures or those of Dean, but it is, apparently, very similar to the condition here described. In *Callorhynchus*, according to Garman's description, "Not far in front of the oral the angular descends towards the mouth from the suborbital. As it nears the lip it takes more of a forward course, and, following near the border of the rostral flap, finds its way down and backward to the edge of the lower surface, where

it turns under and inward to cross the wing and meet the angular of the other side" ('88, p. 75). A very clear idea of the course of this canal cannot be had from this description, and the figures of neither Garman nor Dean, make the matter any plainer. It is evident, however, that the course of the canal is quite different in *Callorhynchus* from what is seen in the other Chimæroids, especially in that it is entirely distinct from the oral and, apparently, from the nasal and subrostral.

The oral canal (figs. 1, 3, *o*) arises from the ventral side of the angular at the point where the latter, as has been said, makes its sudden turn towards the head. It passes ventrad and cephalad, in a gentle curve, like a reversed letter S. Passing under the throat, one or two centimeters caudad to the mouth, it meets its fellow in the mid-ventral line. Like the jugulars, the ventral ends of the orals, where they cross the throat, are broken up into a series of short dashes (fig. 3, *o*), instead of having the continuous, groove-like form seen elsewhere. Along the anterior border of the dorsal curve of the oral may be seen a row of about a dozen pores; they follow the direction of the canal until they reach the labial folds, then they turn cephalad and pass around the anterior end of the head, between the nostrils and the nasal canals (figs. 1, 3, 4), becoming smaller as they pass forward. In *C. monstrosa*, *Rhinochimæra* and *Harriotta* the course of the oral is the same as in the present form, but in *Callorhynchus* it arises from the orbital, just posterior to the angular.

The nasal canal (figs. 1, 3, 4, *n*) arises from the ventral side of the angular in its anterior half. It passes ventrad and cephalad for a short distance and then turns sharply cephalad to meet its fellow in the median line, about half way between the nostrils and the point of union of the angular canals with the median subrostral. Throughout its length the outline of the nasal is broken by a series of enlargements like those already noted in connection with the rostral, suborbital, and angular canals. As has been noted above, Garman calls that part of the angular which lies anterior to the origin of the nasal the subrostral, but there seems to be no reason for so doing. The course of the nasal canal in *C. monstrosa* is, apparently, the same as in *C. coliei*. Its course

in the other Chimæroids could not be determined by the figures of either Garman or Dean.

The subrostral canal (figs. 1, 3, 4, *sr*) has the appearance, when seen in a ventral or an anterior view of the head, of a letter Y. The stem of the Y, and the two branches, are each about five millimeters in length, in a fish of average size. Each branch of the Y begins in an enlargement at the point of union of the rostral and suborbital; it thence passes ventrad and mediad to meet its fellow in an enlargement at the dorsal end of the stem of the Y; this stem which, of course, lies in the median plane, extends ventrad to the enlargement at the point of union of the two angular canals.

In *C. monstrosa* the course of the subrostral is the same as has just been described, except that, if Garman's figures be accurate in this detail, the enlargements are not in the same position. The course of the subrostrals in the other Chimæroids could not be determined from the figures and descriptions at hand.

THE STRUCTURE OF THE CANALS

Although numerous workers have described the structure of the lateral line organs in other fishes, no one, apparently, has described the anatomy of these organs in *C. coliei*; and the work of Solger ('79) and others upon *C. monstrosa* leaves much to be desired.

According to Garman ('88) the chief structural difference between the canals of the *Chimæra* and those of the other genera of the *Holocephali* is that in the former genus they have the form of an open canal, while in the other genera they are tubular in structure. According to the method of development of these canals it would seem, then, that, in this respect, at least, the genus *Chimæra* is more primitive than the other genera of the group.

As viewed from the surface there appear to be two types of canal; the common form that is seen in the lateral line proper (figs. 1, 2, *l*) and most of the cephalic canals; and the form that has the previously mentioned enlargements, and is represented by the canals on the anterior region of the snout (fig. 1). The

former type will be referred to as "type 1," the latter as "type 2." Solger ('79), in *C. monstrosa*, calls these two types of canals "primary" and "secondary" forms, respectively.

As seen with the naked eye the canals of type 1 seem to be wide grooves, bordered closely, on each side, by a dark line; but when examined under a low magnification it is seen that the slit-

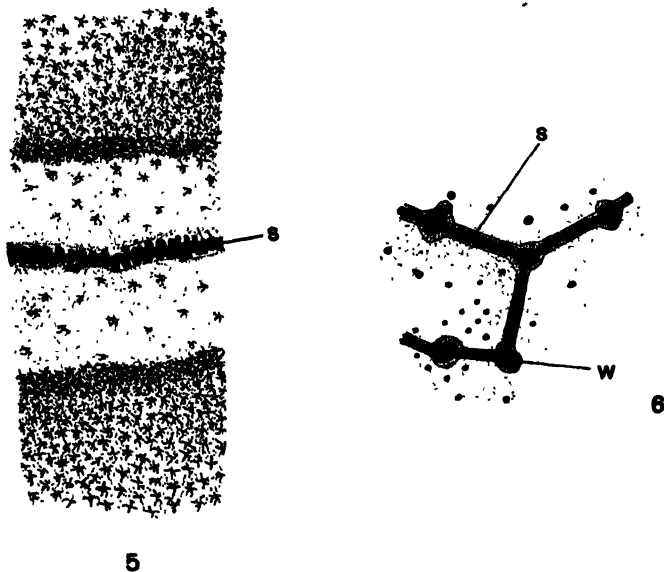


FIG. 5 A surface view of a small portion of a canal of type 1; drawn under a low magnification of the microscope, by reflected light.

FIG. 6 A small portion of two canals of type 2; drawn under a low magnification. Five of the wide spaces (*w*), described in the text, are shown, as well as the shelf, and the round gland openings of the surrounding integument.

like opening of the canal is much narrower than it appeared to the naked eye. That which gives the appearance of a wide opening is a border, on each side of the slit, where there is very little pigment. Beyond this light border there is, on each side, a very densely pigmented area that, at first glance, is taken for the edge of the slit. Fig. 5 represents a portion of a canal of type 1 as seen under a low magnification of the microscope. The dark narrow border on each side of the light area is more distinct when seen

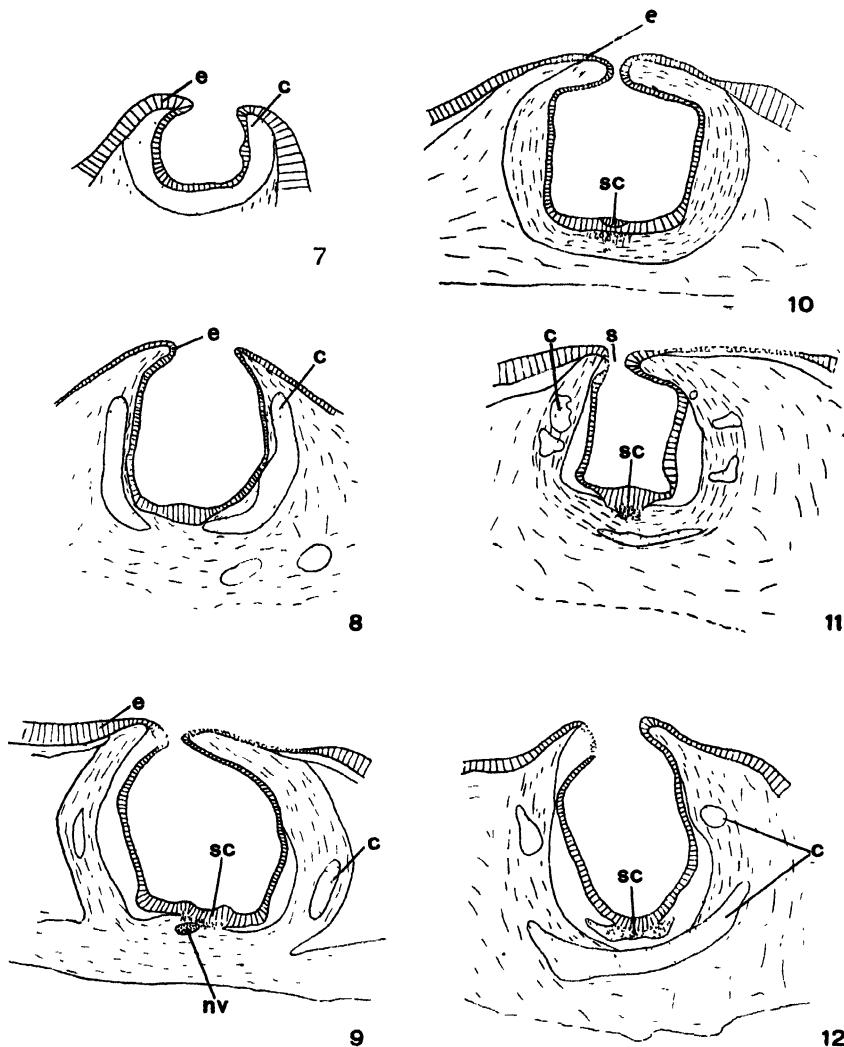
with the naked eye than is shown in the figure. The pigmentation is chiefly due to great numbers of chromatophores having the shape of typical spider or mossy cells.

The actual slit, as seen in fig. 5, *s*, is very narrow, though it may vary somewhat, in different regions. Its outline is very irregular, though this also varies in different regions. The cause of these small, irregular projections will be seen a little later.

The canals of the second type (fig. 6) are five to ten times larger than those of type 1, although, superficially, they do not appear so because of the light border, noted above, in connection with the latter type. As seen with the naked eye or under a lens the canals of type 2 have somewhat the appearance of fig. 6. Around the inner margins of the wide spaces, a short distance below the level of the surface (fig. 6, *w*), is a sort of shelf that partly closes the opening. In some canals this shelf extends along the narrow part of the canal, between the widenings, as is shown in fig. 6. The meaning of this shelf will be explained below. The wide spaces are not always so symmetrical as those shown in the figure; in some places the widening is all on one side of the canal; and in other places, the widenings of the two sides do not lie exactly opposite each other.

The structure of the canal is best made out by the study of transverse sections, but some features can be seen in longitudinal sections, and in pieces of the entire canal that have been cleared for study under the low powers of the microscope. Serial sections were made of eight or ten different regions of the canal system. Both the paraffin and the celloidin methods were used, the latter giving the better results.

Figs. 7 to 14*a* are a series of semi diagrammatic camera-drawings of cross sections of different regions of the system (see Description of Figures). Figs. 14, 14*a* were drawn under a magnification of fourteen diameters, while all the rest were magnified forty-one diameters. It will be noticed that all the sections of canals of type 1 (figs. 7-13) are of about the same size and character (the difference in shape is probably due mainly to fixation), except that the section from the tip of the tail (fig. 7) is much smaller than the



FIGS. 7-13 Transverse sections through various canals of type 1, $\times 41$. Outlines drawn under the camera; the cell structure diagrammatic.

FIG. 7 Lateral canal at the extreme tip of the tail, as shown in fig. 16

FIG. 8 Lateral canal just posterior to the place where it drops ventrad to extend along the edge of the caudal fin.

FIG. 9 Lateral canal in the region of the posterior dorsal fin.

FIG. 10 Lateral canal in the region of the dorsal spine.

FIG. 11 Aural canal, between the suborbital and the origin of the oral.

FIG. 12 Orbital canal, between the lateral canal and the jugular.

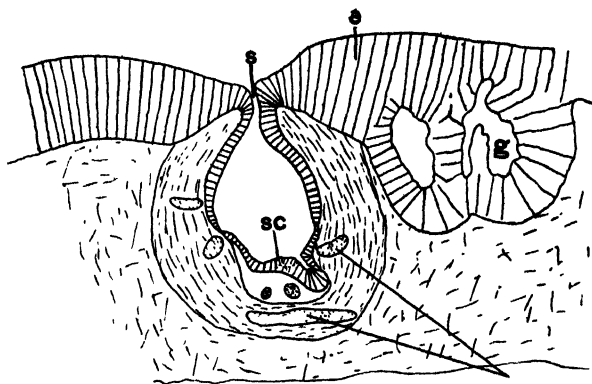
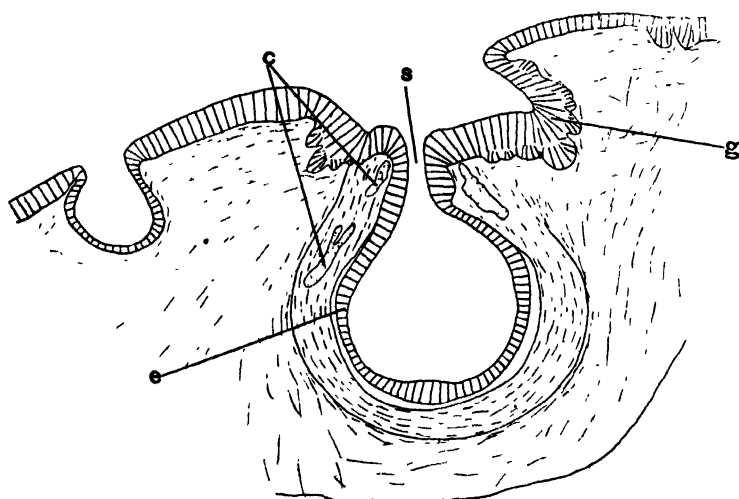


FIG. 13 A section of the rostral canal in its middle region.

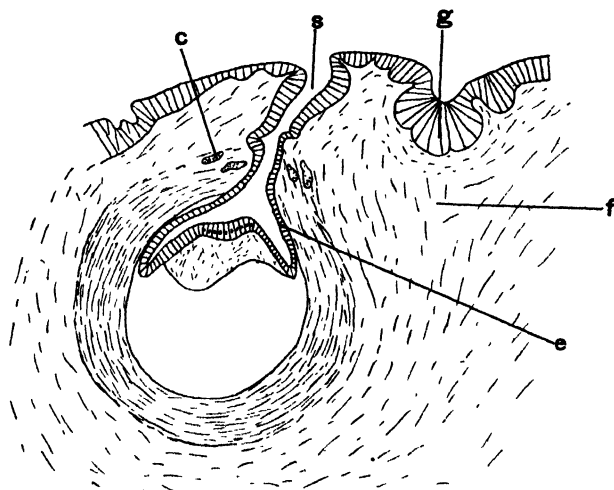
others. The cross section of the canal of type 2 (figs. 14, 14a), as has already been said, is very much larger than that of type 1.

The most striking feature, perhaps, of both types of canal is the cartilaginous wall, which consists of a close-set series of cartilaginous or fibrous rings ("osseous supports" Solger ('79) calls them) united by less dense fibrous tissue. These rings serve to prevent the collapse of the canal. The rings are not at right angles to the long axis of the canal, but form an angle of 60–70 degrees with this axis. At the bottom of the canal the rings are distinct from each other, and are separated by areas of less dense fibrous tissue of about the same width as the rings. As they pass up around the sides of the canal the rings split up into a number of small branches. These branches spread out and form a sort of network at the top of the canal. It is the ends of these branches that make the margin of the opening of the canal so irregular, as was noted in speaking of the superficial appearance of the canals of type 1. The branches of the adjacent rings meet, but apparently do not fuse with each other; they spread out, to some extent, in a vertical as well as in a horizontal direction. The arrangement of the rings is shown diagrammatically in fig. 15. The inclination of the rings from the vertical is seen best in longitudinal sections.

If entire pieces of the canal be viewed by transmitted light, under low magnification, the rings appear as a series of dense



14



14 a

FIG. 14 and 14a. Sections of the aural canal (type 2) in the region of the nasal. $\times 14$. Outlines drawn with camera; cell structure diagrammatic.

FIG. 14 Section through one of the wide spaces described in the text.

FIG. 14a Section taken between one of the wide spaces.

bands separated by more transparent areas of about the same width. Because of their inclination, transverse sections rarely, if ever, show the entire circumference of the rings.

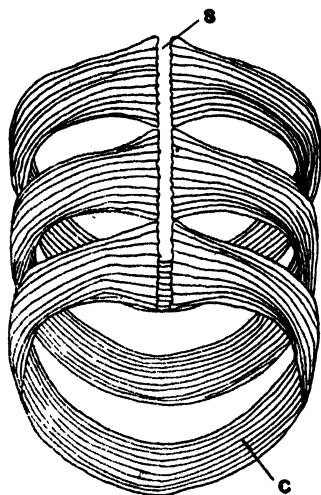
In *Rhinochimæra*, as well as can be judged from Garman's figure ('04), the fibrous rings are not completely separated from each other by other tissue, and they break up into smaller branches much nearer the bottom of the canal than is the case in *C. collei*.

In the extreme tip of the tail the animal is so compressed laterally that the fibrous rings, in spite of their small size are practically in contact with each other in the median plane (fig. 16). In other parts of the system the rings are surrounded by the more or less dense mass of fibrous tissue of the dermis.

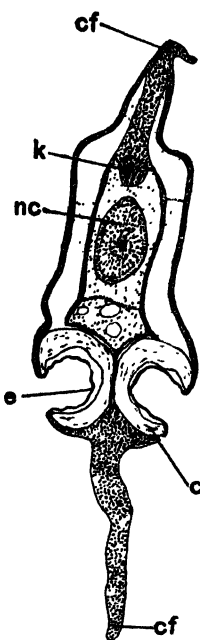
The shelf that has already been noticed, in connection with the canals of type 2, is seen, in cross section (fig. 14, *g*), to be due to a large group of glands, arranged, mainly around the periphery of the wide spaces in the canal. What the character and purpose of the secretion of these glands may be could not be determined. In some regions of the canal this secretion seemed to almost fill the lumen, while at other places it was practically wanting.

The lining epithelium of the canals, which is especially modified at certain regions to form the sense organs, is directly continuous with the outer layers of the epidermis of the body integument.

The sensory epithelium of the first type of canal will be described first. The epidermis of the body integument varies considerably in thickness, but where it turns inward to form the lining of the canal it becomes much thinner, usually with some suddenness. Owing to unsatisfactory fixation, as has been mentioned above, the minuter structures of the lining epithelium could not be determined, especially in the parts of the canal nearer the opening. In these regions of the canal the epithelium lies close to the cartilaginous and fibrous rings, to which it is united by a thin layer of less dense fibrous tissue. This layer seems easily torn, for the epithelium is, in many cases, torn away from the surrounding rings. The epithelium here consists, as nearly as can be determined, of a few layers of small, irregular cells—a sort of transitional epithelium. As the bottom of the canal is approached this epithelium, especially in certain regions, to be noted shortly, suddenly



15



16

FIG. 15 A diagram to illustrate the arrangement of the cartilage rings of the canal, described in the text.

FIG. 16 A transsection through the entire animal near the tip of the tail.

thickens and passes across the floor of the canal as a stratified epithelium of ten or more layers. Along the median line of the floor of the canal this thickened epithelium becomes modified to form the sense organs. These sense organs are segmentally arranged, but there is, apparently, no very sharp line to be drawn between the ordinary epithelium of the floor of the canal and that which is modified into sensory epithelium.

At intervals of about one to two centimeters the epithelium of the floor of the canal is especially thick and is modified to form the sense organs, while between these organs the epithelium is of varying thickness, at the thinnest being but little thicker than that of the outer parts of the canal described above. Between the epithelium of the bottom of the canal and the fibrous rings is

a considerable layer of loose fibrous tissue in which one or more small nerve trunks (figs. 9, *nv*) may be seen.

Fig. 17 represents a transection of the epithelium and underlying nerves and connective tissue, from the floor of a canal of type 1, through one of the regions of greatest modification. The stratified epithelium (*e*) rapidly thickens, as it approaches the middle line, and is suddenly changed into the mantle cells (*ms*), which are vertically arranged, fiber- or rod-like cells, in which nuclei can with difficulty be seen. These mantle cells are bent over towards the median line of the canal to almost inclose the conical mass of sense cells (*sc*).

While the minute details of the sense cells could not be determined, they are, apparently, of about the shape of the sensory cells seen in the taste buds of the mammalian tongue. Each cell has the form of a much-elongated cone, ending in a sensory hair or bristle. A group of these bristles is seen, in fig. 17, *b*, projecting freely between the edges of the mantle cells. Each cell contains a large, oval nucleus with a distinct nucleolus. Whether or not there are elongated supporting cells between these sensory cells could not be determined. At the base of the sensory cells is a mass of small irregular cells (*sp*) which may correspond to supporting cells; they may send fine processes up between the sensory cells but no such processes could be seen.

As has been said above this group of sensory and mantle cells is larger and more distinctly seen at intervals of one to two centimeters but it is not a sharply defined sense group, like a taste bud. It becomes gradually smaller as it is followed caudad or cephalad from the section represented in the figure, until it practically disappears; then it becomes gradually larger again.

As might be expected in poorly fixed tissue the fine connections between the branches of the lateral nerve and the nerve cells could not be determined.

The groove-like depression in the upper side of the sensory epithelium, into which the bristles extend, seems to be invariably present.

The epithelium of the canals of type 2 is quite different from what has just been described. These canals, as is shown in figs.

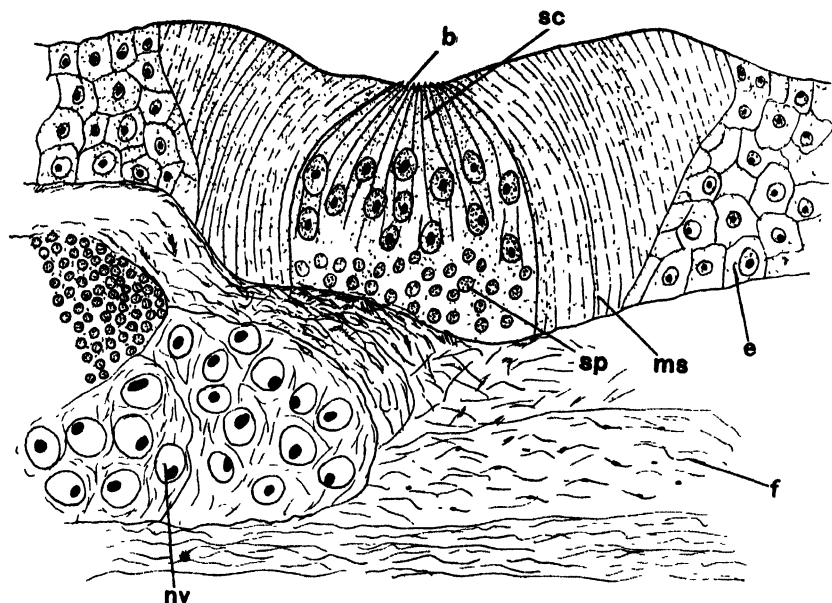


FIG. 17 A drawing, made under high magnification, of a section through one of the sense organs of a canal of type 1. Only a small portion of the epithelium near the median line of the canal is shown.

14 and 14a lie further below the surface, so that, in the regions between the enlargements, a cross section has the appearance of a simple alveolar gland, with a duct of considerable length (fig. 14a).

As the stratified epithelium of the surface extends down into this deep groove it becomes reduced in thickness until, in the region where it passes between the upper ends of the cartilage rings, it consists of only a few layers of cells. At this point the superficial cells of the epidermis are suddenly converted into a layer of rather short columnar cells: while the lower cells of this epidermis become continuous with (though they could scarcely be said to be converted into) the thin layer of loose, fibrous tissue that lies between the epithelium of the canal and its cartilaginous and fibrous rings. This transition from the superficial to the lining epithelium is quite sudden and is fairly distinct, even in the tissue at hand.

In the wide regions of the canals the superficial epithelium above the cartilaginous rings is modified to form the glands (fig. 14, g), that have been mentioned, but the transition into the columnar

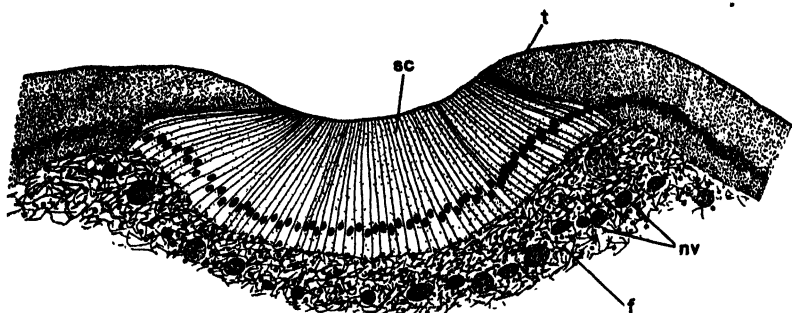


FIG. 18 A section of sensory epithelium of a canal of type 2; corresponding to that shown in the preceding figure.

epithelium is the same as has just been described. In this region the columnar epithelium retains about the same character around the entire periphery of the canal, being scarcely any thicker along the floor than in the more distal regions. This is shown, diagrammatically, in fig. 14, *e*.

In the part of the canal (of type 2) between the wide spaces there is some differentiation in the columnar epithelium. This differentiation is greatest in the middle regions and disappears gradually as the wide spaces are approached. In this middle region, between the wide spaces, the epithelium gradually becomes thicker as it passes from the slit to the floor of the canal, until, along the median line of the floor, it is about twice as thick as at the opening. This greater thickness is due to the greater length of the columnar cells.

Along the median line of the floor of the canal is seen the same wide, shallow groove that was noticed in connection with canals of type 1 (figs. 14*a*, 18). The cells that form the bottom of this groove are quite distinct from the rest of the epithelial lining of the canal, and would seem to represent the sensory epithelium, though they possess no bristle-like ends, and no nerve connections could be made out. They are simply long, tapering, columnar cells, each with a darkly stained nucleus near the basal end. They stain less strongly than the cells on either side, so that they are always distinctly differentiated from them, as is shown in fig. 18, *sc*. The surrounding cells are, perhaps, narrower than those just described, and become shorter as they pass outward and

upward towards the opening. They do not form the special group of mantle cells, noticed before in connection with the other type of canal; their sharp differentiation from the median cells is mainly due, as has been said, to different staining properties. The free ends of all the columnar cells have a narrow, dark border, apparently a top-plate, *t*. Beneath the median cells is a fairly compact mass of fibrous tissue, *f*, through which are scattered numerous dark bodies, which seem to be small nerve trunks and individual nerve fibers, *nv*.

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EXPLANATION OF THE LETTERING

<i>an.</i> , angular canal.	<i>ns.</i> , nostril.
<i>au.</i> , aural canal.	<i>nv.</i> , nerve.
<i>b.</i> , sensory bristle.	<i>o.</i> , oral canal.
<i>c.</i> , cartilage ring.	<i>oc.</i> , occipital canal.
<i>cf.</i> , caudal fin.	<i>or.</i> , orbital canal.
<i>cr.</i> , cranial canal.	<i>pa.</i> , post aural canal.
<i>e.</i> , epithelium.	<i>r.</i> , rostral canal.
<i>f.</i> , fibrous tissue.	<i>s.</i> , slit-like opening of canal.
<i>g.</i> , gland.	<i>sc.</i> , sense cells.
<i>j.</i> , jugular canal.	<i>s.</i> , suborbital canal.
<i>k.</i> , spinal cord.	<i>sp.</i> , supporting cells.
<i>l. & l'</i> , lateral canal.	<i>sr.</i> , subrostral canal.
<i>m.</i> , mouth.	<i>t.</i> , top plate.
<i>ms.</i> , mantle cells.	<i>w.</i> , wide space of canal.
<i>n.</i> , nasal canal.	<i>y.</i> , y-shaped branch of occipital canal.
<i>nc.</i> , notochord.	

ON A NEW RHABDOCÆLE COMMENSAL WITH MODIOLUS PLICATULUS

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FORTY-ONE FIGURES

During the summer of 1909, while searching for sporocysts and rediæ in the mollusks of the Woods Hole region, at the Bureau of Fisheries Laboratory, I found an interesting turbellarian in the ribbed mussel which is not only new to this continent but which possesses some characteristics which seem to mark it as unique among the Rhabdocœlida.

The first specimen encountered was taken to be a redia, but later when it was noted that not only was the worm ciliated, but that it contained ciliated young, it was seen to be a viviparous rhabdocœle. After much experimenting it was found that the worms could be collected to the best advantage by opening the shells of the mussels and shaking the animals about in a dish of sea water. They are thus washed off the gills and will be found creeping about on the bottom of the dish. Much time was spent in looking over the gills and mantles of mussels in the hope of finding some of the worms in place; none were found, however, on any part of the animal. This is not to be wondered at when the small size of the worms is considered, and further, that they are practically the same color as the gills. Indeed it happened more than once that what was thought to be one of the worms proved to be a small piece of gill or mantle which had been torn off by accident and was moving about in the water by its own ciliary action.

The species belongs to the genus *Graffilla* or to a closely related genus, but is quite different from any species noted by von Graff in his *Monographie der Turbellarien*.

DESCRIPTION OF THE SPECIES

Graffilla gemellipara sp. nov.

Small, rarely reaching 2 mm. in length; nearly linear, bluntly rounded at the extremities, often round-pointed at the posterior end, frequently bent a little to one side, making the outline arcuate, rather thick; ciliate throughout; color white, often tinged with yellow or greenish-yellow along the middle line; mouth small, ventral, near the anterior end; pharynx subglobose; esophagus short; intestine very difficult to distinguish in living specimens, extends along the dorsal side to the posterior end, its limits not always well-defined; eyes two, black, reniform, with a well defined "brain" behind them; genital opening very small, ventral, situated at about the anterior third. There is a pyriform seminal vesicle which communicates with the genital pore by a short duct in which lies a plug-like penis. The ovaries are two ribbon-like bodies placed laterally and beginning about the anterior fourth and extending to the middle of the body. The yolk-forming cells (vitellaria) appear to be continuous with the ovary and are widely distributed. In all the larger specimens there were numerous young in various stages of development. The fully developed young, as a rule, lie together by twos inside a thin capsular envelope. They are ciliated and very active, turning round and round within the capsule, or, after breaking through the capsule, making their way with ease through the mesenchyme of the parent. In the larger worms there are usually a number of small, irregularly coiled string-like bodies scattered throughout the body which have the appearance of being secretion products (figs. 16, 17). At first the idea that they are either of the nature of giant rhabdites or spermatheca was suggested. They appear to be collapsed embryonic capsules from which the embryos have escaped. Some specimens were collected which were immobile and contained but little beside the numerous active young. The latter are evidently not liberated until the reproductive powers of the mother are exhausted when they make their escape through the ruptured body-wall. Dimensions, in millimeters, specimen slightly flattened; length 1.54; breadth, maximum, 0.65; breadth

at eyes 0.28; distance between eyes 0.10; distance of eyes from anterior end variable, but most of the time about 0.14; pharynx nearly circular in outline and about 0.09 in diameter; ciliated young very variable in life, on account of contraction changes; but usually 0.12 to 0.14 in length; diameter of a capsule containing a pair of young 0.11. A small specimen without young, slightly compressed, measured 0.56 in length and 0.32 in breadth, and the diameter of the pharynx was 0.04. A large specimen, which was in a moribund condition, semi-transparent, turgid and devoid of cilia, measured 1.75 in length, 0.37 in breadth at the eyes, 0.63 at the middle, and 0.28 near the posterior end.

NOTES ON THE ANATOMY OF THE SPECIES

It is not my purpose to give histological details; indeed, my material, although consisting of numerous whole mounts and a number of series of sections, while enabling me to understand much of the anatomy, still leaves much unexplained.

Epidermis, etc.

The epidermis is ciliated, the cilia appearing in the embryos before the pigmented eye-spots are seen. The cells are irregularly cubical with large nuclei. In cases where the epidermis was seen in living specimens crushed under the cover-glass the outlines of the cells were very irregular. Four sets of muscle fibers can be distinguished in sections from material which was fixed in Flemming's chrome-osmo-acetic fluid and stained in iron hæmatoxylin. These are longitudinal, transverse, and two sets of diagonal fibers (fig. 15). There are no rhabdites, and I fail to recognize the subhypodermal glands which are said to be present in the parasitic turbellarians.

In the living worm, under favorable conditions, there may be seen a reticulum of slender strands uniting here and there into masses of similar substance, the whole resembling a plexus of fine nerve-fibers with interspersed ganglia (figs. 13, 14). No nuclei were observed in these masses. The strands are solid, and

I take them to represent a subdermal nerve plexus. They lie in the body-wall, probably closely associated with the muscular layers. This was evident in the living and active specimens. When the worm was contracting actively and the mesenchyme was thrown into constant motion so that the various structures which were lying in it, even such stable bodies as the pharynx and eyes, were constantly shifting their position, the reticulum remained unchanged and moved only with those movements of the worm which involved the outer body wall

Alimentary tract

The mouth, although very minute, may occasionally be seen in specimens that are lying free in sea water and viewed under low magnification. When the worm, in making its characteristic movements described below, turns the head sharply to one side, a view of the ventral surface in profile is often obtained. A notch in the outline of the ventral surface indicates the position of the mouth. This position is somewhat variable on account of the extreme mobility of the body-wall. In one that was watched for some time there appeared to be a longitudinal furrow leading from near the level of the anterior border of the pharynx to the anterior end of the body. The sides of this furrow were capable of being pressed together, thus extemporizing a gullet (fig. 5). The mouth proper appeared to be near the anterior edge of the pharynx. It was variable in shape, usually ovate with the larger end in front; sometimes it was circular. The furrow and the mouth are, of course, ciliated, as is the whole surface of the body.

The pharynx is near the anterior end and is subglobular. In the living worm it was seen performing swallowing movements, and in one case where the pharynx had been entirely separated from the body in a crushed specimen it continued to contract convulsively for some minutes.

The esophagus is short, scarcely as long as the pharynx. In a horizontal section measuring 1 mm. in length, the pharynx was 0.05 mm. from the anterior end, and measured 0.07 mm. in diameter, and the esophagus was about 0.05 mm. in length. The

transition from the esophagus to the intestine is not abrupt. In the case cited it maintained about the same diameter for a distance of 0.05 mm., then widened rapidly for about the same distance into the intestine (fig. 4).

The intestine extends to the posterior end of the body. Its position is dorsal. In life the cells that line its cavity seem to be rather loosely attached. They are usually yellowish or yellowish-green, especially in the older individuals. Rudiments of the esophagus and pharynx were noticed in young individuals that were still in the embryonic capsules. The intestine is usually rather difficult to see in the living specimens. In one instance it was plainly seen as a thin walled elongated sac extending nearly to the posterior end. It was filled with yellowish granules suspended in a fluid, and the contents were kept moving backward and forward by the contractions of the body. The specimen being under slight pressure, some of the intestinal contents were pressed out of the mouth.

Male genitalia

I have not yet been able to make out the anatomy of the genitalia with entire satisfaction. This apparent indefiniteness of the genital organs may indeed be incident to the viviparous condition, which may, in turn, be seasonal and parallel with the production of summer eggs as has been shown to be the case with some of the Mesostomata. It is more probable, however, that the species is protandrous.

The genital pore is ventral and approximately at the anterior third or fourth. In one specimen which, flattened under the cover-glass, measured 2 mm. in length, the genital pore was approximately 0.06 mm. from the anterior end. In another, measuring 1 mm., it was 0.25 mm. from the anterior end. Sections show that the pore communicates with a subglobular or pyriform sac which contains spermatozoa. These were seen in active motion within the sperm-sac in living specimens. There is a short penis which lies like a plug in the duct leading from the

sperm-sac to the genital pore. In living worms it was noted that the sperm-sac often lay behind the eyes on the middle line in such position that it and the eyes formed an equilateral triangle. The sac is usually pyriform. Inside dimensions of a living specimen: length 0.048; breadth, at dorsal end 0.051, at ventral end 0.034. In this case it was noted that the walls of the sac were clearly limited on the inner side, but not so clearly defined on the outer side. Its walls are rather thin, but they become thick and muscular where they merge into the penis sheath. In life the sac is actively contractile. On one occasion a curious behavior of the sperm-sac was noticed. An ovum, with two large, clear germ cells embedded in a mass of yolk, lay just behind the sac and touching it. The ovum was constricted in the middle where it was in contact with the sac and the two germ-cells had passed one to each side of the sac (figs. 37, 38). At the posterior border of the sac there was a wisp of fibers as though sperm were being forced into the ovum. At the same time there was an almost rhythmical contraction of the posterior border of the sac. The ovum was also contracting somewhat rhythmically. In another specimen the sperm-sac, which was collapsed and pushed to one side, appeared to be trying to approach one of the densely granular ova which was lying adjacent to one side of the worm. This ovum had one large, clear germ-cell, and was actively contractile. The impression made on the mind by this behavior was that something of the nature of copulation was in progress (fig. 39). This singular action did not appear to be due to pressure of the cover-glass. In one specimen a cluster of spermatozoa was noticed on the median line a short distance behind the sperm-sac. The genital pore lay between the sac and the cluster of sperm. Similar clusters of sperm were seen in sections in a duct which lies posterior to the sac and leads dorsad to the yolk-forming cells of the ovary (fig. 40).

The testes were not satisfactorily made out. In a series of horizontal sections, made from a specimen which had been fixed in corrosive-acetic and stained with hæmatoxylin and orange G, branching glands near the dorsal surface (fig. 8) were at first taken to represent the testes, but are evidently vitellaria. In a young

specimen two small, subglobular bodies were seen a short distance back of the pharynx and laterally placed. These may be testes.

Female genitalia

The ovaries are elongated, compact masses of cells lying, for the most part, lateral and ventral to the intestine. A large part of the ovary consists of characteristic cells elongated transversely to the length of the organ, with abundant, very fine-grained cytoplasm, and large, clear nuclei with conspicuous nucleoli. These cells enlarge, gather a very large amount of yolk, ultimately become detached, and are then driven about in the mesenchyme by the movements of the worm. In horizontal section (figs. 40, 41) the two ovaries are seen to be continuous on the middle line on the ventral side of the intestines with smaller cells which are more coarsely granular than the cells of the ovary and have smaller nuclei. A duct, traced from the genital pore to the middle of this gland, seems to answer to the uterus, but spermatozoa were found in it, and, moreover, there appeared to be two ducts in the vicinity of the yolk-cells. It is possible that a series of sections of a young specimen might show that there is a duct leading from the dorsally-placed testes to the sperm-sac. I have not succeeded in demonstrating the way in which the spermatozoa reach the sperm-sac. It will be noticed from the foregoing account that there is some reason for thinking that the cells which are liberated from the ovary into the mesenchyme may be fertilized by sperm from the sperm-sac, which, if that be the case, would then be a sperm receptacle filled from another individual. I find nothing in my sections to warrant this conclusion, and nothing in the stained and mounted specimens except one curious case of an ovum and sperm-sac in close proximity (fig. 38).

DEVELOPMENT

Many stages of development, from the large yolk-burdened ovum with a single nucleus to the ciliated young, may be seen at the same time in the same adult worm. Some of these forms are

figured (figs. 1 to 4, 7, 20 to 30, 37 to 39, 41). It would extend this paper beyond its intended scope to describe them in detail. Briefly the normal course of events in the development of the young seems to be as follows:

1. The egg as it is liberated from the ovary-vitellarium is characterized by having a relatively large amount of yolk in which there is a large, clear nucleus with a distinct nucleolus (fig. 1, *h*). How the capsule is acquired was not made out

2. The nucleus divides, and sooner or later there is a cleavage of the resulting mass of cells into two divisions; or, the two nuclei may separate at the completion of the first segmentation (fig. 3, *f* and *b*). So far as I have been able to interpret the evidence, the embryos which are enclosed in the same capsule seem to have developed from the same ovarian ovum.

3. Each division is soon seen to be made up of two kinds of cells. (*a*) Small and numerous, massed at one pole, which ultimately becomes the head of the young worm. There is also a narrow layer of these small cells around the periphery (*b*) Large, globular cells, relatively few in number. These lie in the posterior region, and continue with little change in the young worms (fig. 41, *e* and *d*). They are probably yolk-cells.

4. The small peripheral cells soon give rise to a ciliated epithelium. A body-wall about as thick as that of the adult appears, pigmented eye-spots are developed and the rudiment of a pharynx is seen. The egg-membrane persists as a capsule in which the pair of young worms are confined long after they are capable of active movements. At first they lie parallel, with their heads and tails in corresponding positions. Later the head of the one is usually beside the tail of the other. They keep up a constant movement round and round, sometimes in the same direction, but often moving independently of each other. Usually the two young and the fluid in which they are immersed are the sole contents of the capsule; at times there may be one or more masses of yolk also included in the capsule. There seem to be individual characteristics in this respect, the course of development of the young in one adult being quite different from that in another (figs. 2 and 3). A few cases were noted where there were three em-

bryos instead of two in the same capsule (fig. 28). So far as such cases have been studied it appears that two of the three are alike and smaller than the third, which should be the case unless the original division of the first cell or the mass of cells were three-fold, an occurrence certainly not to be expected. Often a young worm is seen not accompanied by its twin. These cases, as a rule, are the result of the rupture of the embryonic capsule and the escape of the young into the maternal mesenchyme where they can wander about freely. It happens, however, that, while it is the rule that two embryos develop in each capsule, only one may at times develop. This is the interpretation which I think must be given a case where a single young worm is still enveloped in the capsular covering (fig. 1, *n*).

The cleavage of the mass of cells into two masses is preceded by the arrangement of the large and the small cells in different ways. In some cases the small cells are at the opposite poles and the large cells in the middle in which case the division takes place through the mass of large cells (fig. 1, *r*). Or the large cells may be at the opposite poles and the small cells in the middle, the division then taking place through the latter (fig. 3, *e* and *f*). In other cases it would appear that at the first division of the germinal spot, the resulting cells have moved apart, each building up a morula-like mass of cells. These two masses of cells remain for a long time separated from each other by an intervening mass of yolk (fig. 22). Such cases as young worms with one or more masses of yolk in the same capsule are thus explained. Some cases were observed where there was nothing but a mass of large cells within a capsule, the yolk having been entirely absorbed (fig. 25). What is the ultimate fate of such cell masses I do not know.

While I did not contemplate the study of such minute structures as nuclear elements, some of the material was prepared with considerable care. Chromosomes are clearly defined, and a few very large centrosomes were noted. What was interpreted to be a polar body was noted in one case. This was a cell which lay inside the egg capsule at the junction between the approximated anterior ends of the embryos.

MOVEMENTS

The behavior of these worms is highly characteristic. When placed in sea water they creep about on the bottom of the dish. They move by means of cilia, but the body in addition is in constant motion. The mesenchyme is soft and yielding and responds to every contractile movement of the body-wall. Although actively moving, the worm does not make progress in any one direction save for a very short time. For example, it will move forward for a short distance, usually not more than its own length. It will then turn the head end sharply to one side or the other. The direction of progress being thus changed, the worm will move in the new direction again for about its own length when, in the same manner, a new direction will be taken. The result is that but little progress is made unless some factor, such as gravity or light, is added. For example, if the dish is not level the worm will be found in the course of a half-hour or so to have changed its position from one side of the dish to the other. While they do not react immediately in any marked degree toward the light, in general the adults tend to move away from it. Thus they may be found to have crept up the side of the dish and be clustered near the surface of the water in response to the action of light. In order to determine whether these worms react in any way to light the following observations were made. A number of them were left over night in a watch-glass. On the following morning they were, so far as I could see, as active as they had been on the previous day. They had all gathered at the side of the dish that was turned away from the light. The side of the dish next the window was then darkened and the opposite side illuminated. In a few minutes the worms were clustered at the darkened side of the dish. The worms do not move in a straight line away from the light, but keep up their habit of starting off in a great hurry for some place, but going only a length or two in any one direction, then turning abruptly to the right or left and thus proceeding by a series of zig-zags. The result of this peculiar method of movement in its natural habitat must be to afford the animal a constant change of position, but as a rule within a limited area.

This would appear to be in harmony with its natural surroundings. If its instinct led it to make long excursions in a straight line the chances of its being carried away from its host would be much greater than they must be with its actual hesitating manner of progression.

The test for the reaction to light was repeated with the same general result. That is, they tend to move away from the light. This instinct, again, it would appear, is what should be expected of a worm living as a commensal within the mantle-cavity of a mussel.

The ciliated young within the embryonic capsule are very active. They are constantly swimming about, and, on account of the confined space in which a pair is obliged to move, the result of the ciliary action alone would be to drive them round and round. Beside the ciliary motion there are frequent changes of direction effected by turning the anterior end of the body to one side or the other. Indeed the characteristic habits of motion of the adult can be detected in the young before they have escaped from the capsule. When the young break through the capsular wall they wander freely in the mesenchyme. Thus they were observed in all parts of the body pushing their way industriously, but never continuing in a straight course for long. They were seen even lying between and in front of the eyes, and indeed in every part of the body, but most of the fully developed young are found in the posterior third of the body of the mother. When the fully developed young escape from the body of the mother their behavior in the sea water is essentially like that of the adults. Many free individuals were collected which were but little further advanced in development than some which were still in the embryonic capsule. On one occasion a young worm was observed pushing its way through the dense median mesenchyme. When it reached the edge of the denser portion it pushed for an instant as if it had encountered something which was resisting its progress. The obstruction gave way suddenly and the worm passed quickly into the thinner mesenchyme which lay along the anterior margin. It is probable that the worm had strayed into the lumen of the intestine and was seen forcing its way out through the intestinal wall.

DISTRIBUTION

This species was found only in *Modiolus plicatulus*. Their geographical distribution is somewhat irregular. It was soon noticed that mussels from some localities had none, while those from other localities yielded them in considerable numbers. Even two mussel beds which were near together might differ very markedly. For example, on August 10 I examined two lots of mussels from Ram Island on which I made the following notes:

1. From the N.W. side near an old wreck, 36, measuring from 55 to 75 mm. in length, most of them about 65 mm., above low tide in a kind of sedge, shells much corroded. No parasites found.
2. From a point 50 yards south of the locality of the first lot, 34, 35 to 75 mm., mostly about 55 mm., above low tide, sandy mud, about 75 parasites obtained.

In general it may be said that mussels in confined coves do not have these parasites. The best localities for finding them are those which are exposed to rather free tidal currents. When they do occur in a bed of mussels they are rather more numerous in the large animals than they are in the small ones.

Advantage has been taken during the process of publication of suggestions made by my friends Drs. Coe and Patterson, both of whom have shown a lively interest in this case of apparent polyembryony.

Dr. Coe writes me that he examined a lot of ribbed mussels from Savin Rock, New Haven, on Oct. 7, 1910, and finds the *Graffilla* fully as common as at Woods Hole. There was, moreover, an abundance of embryos in all stages in the larger specimens.

TABLE I

The following notes will show further the somewhat uncertain distribution of this worm.

DATE	LOCALITY	NUMBER OF MUSSELS	SIZE	NO. OF PARASITES
July 12	Ram Island	50	45-65	14
July 12	Ram Island	50	25-35	8
		13		
July 15	Ram Island	225	30-75	229
July 16	Ram Island	49	45-80	9 large, 6 small
July 16	Sheep-pen Cove	77	40-85	0
July 20	North Falmouth	100	35-60	0
July 29	Katama Bay	30	large	0
July 30	Head of Great Harbor	86	large	0
August 4	Robinson's Hole	78	55-98	0
August 5	Wareham	84	50-70	10
August 7	Ram Island	63	35-68	110
August 10	Ram Island	36	55-75	0
August 10	Ram Island	24	35-75	75
August 27	Cuttyhunk	4	63-110	5
August 27	Hadley Harbor	79	45-70	0
August 30	Ram Island	80	32-75	204

TABLE II

The following tabular statement shows the results of the examination of a lot of 225 mussels divided according to size.

NO.	SIZE	NO. OF PARASITES AND REMARKS
25	60-75	45, nearly all large.
25	50-58	7, large and small.
25	50-58	42, large and small.
25	45-50	16, large and small in about equal numbers.
25	40-45	22, more small than large.
25	35-40	28, mostly minute.
25	32-35	30, mostly minute.
25	30-32	17, all minute.
25	30	22, 2 or 3 small, others minute.

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EXPLANATION OF FIGURES

PLATE 1

EXPLANATION OF FIGURES

1. Sketch of specimen, flattened and mounted in balsam; dorsal view; actual length 1.43 millimeters. *a*, pharynx; *b*, esophagus; *c*, eye; *d*, sperm sac; *e*, *e*, embryo consisting of a central mass of cells surrounded by a yolk; others will be found to which no index lines point; for example, there is one almost exactly in the middle of the body and another just behind the embryo to which the index of *f* points. The latter appears to be on the point of dividing. A similar stage of development will be found in front of *r*. *f*, embryo with large cells at opposite poles and separated by yolk; another will be found on the median side of the sperm vessel, while between the latter and the anterior end of the left ovary is one which is a little farther advanced. *h*, ovum with a large germ cell enclosed in yolk; *k*, collapsed embryonic capsule; another is shown just behind *n*; *l*, twin embryos enclosed in the same embryonic capsule; others are shown in various parts of the body; *m*, yolk cells; *n*, single young in capsule; *o* left ovary; *p*, mass of cells in capsule, all the yolk is absorbed; *q*, early stage of twins enclosed in yolk; *r*, this represents a stage in development just before division into two masses of cells; *z*, young escaped from capsule and wandering about in the mesenchyme.

2. Sketch of specimen which had been killed under slight pressure over flame and was lying in sea-water. *a*, two large cells with coarsely granular yolk; *b*, twins, ciliated, but the eyes have not yet appeared. In the anterior capsule there are a few yolk granules which have not yet been absorbed. This is also the case in the capsule median to *a*. In the posterior one all the yolk has been absorbed. *c*, twins, ciliated and with eyes. There are three other pairs shown; it is to be noted that in each case some of the yolk has not been absorbed.

Actual length 1.61 millimeters.

3. Another, also in sea water; actual length 1.30 millimeters. *a*, brain; *b*, ovum with a germinal cell at each end separated by yolk; *c*, sperm sac; *d*, probably a part of the ovary distorted by pressure; *e*, embryo consisting of two masses of large cells separated by yolk and small cells. This form has originated from a condition like that represented in *b*; it is about ready to separate into a pair of twins. *f*, this shows the division completed which is faintly indicated in *e*. Another representing a similar stage is just behind *f*. *h*, a pair of twins, ciliated but eyes not yet developed; *j*, twins ciliated and with eyes. It will be noticed that all the young in this specimen have divided in such a way as to include the yolk. *k*, a granular body, probably an ovum in which the nucleus is concealed by the mass of yolk.

4. Longitudinal, horizontal section *a*, pharynx; *b*, esophagus; *c, c*, intestine; *e*, segmenting ovum similar to *f*, fig. 1; *f*, segmenting ovum, large cells at opposite poles; *j*, *k*, *l*, *m*, ciliated embryos; *o*, ovary. Length 0.97 millimeter.

5. Ventral view of head showing mouth and a preoral furrow; sketched from life.

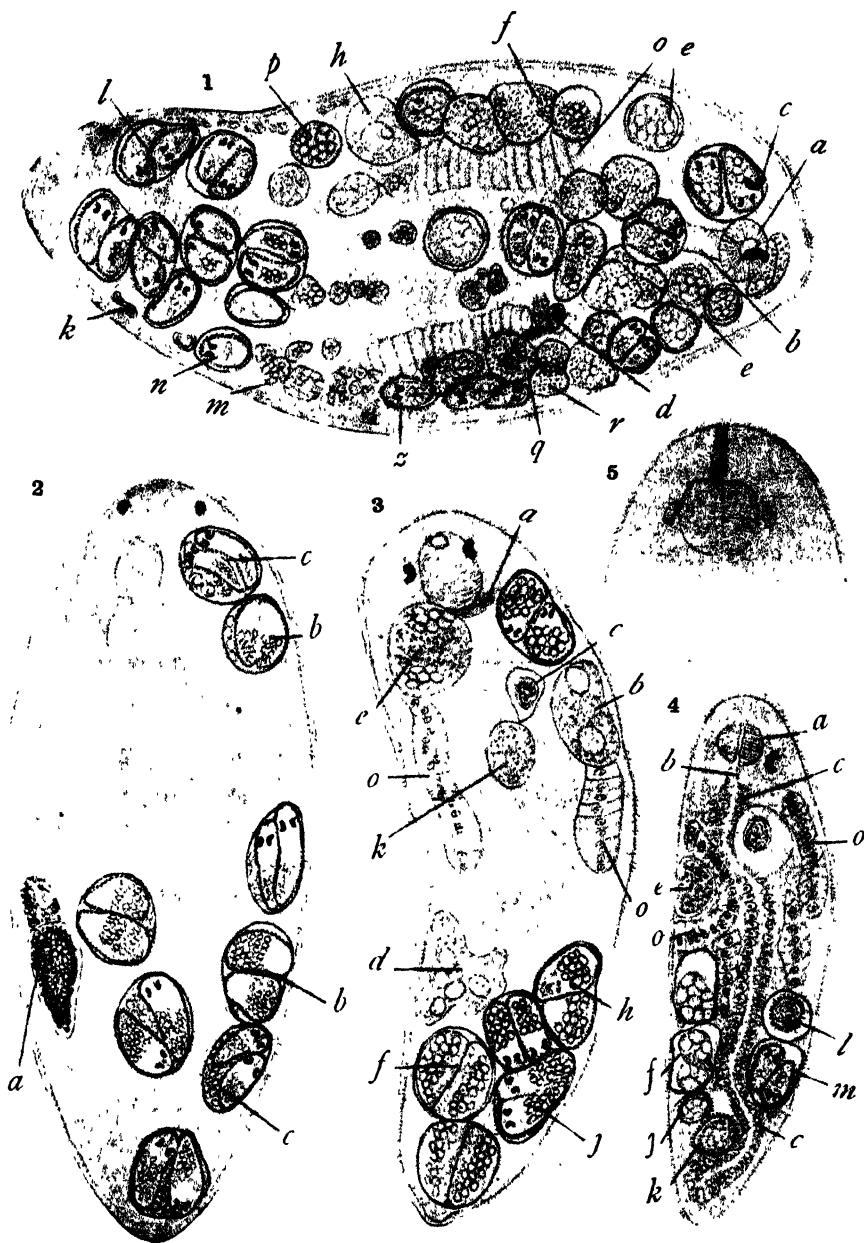


PLATE 2

EXPLANATION OF FIGURES

6. Horizontal section showing brain; actual diameter in region of the eyes 0.17 millimeter. *a*, pharynx; *b*, brain; *c*, muscular layer; *d*, cells in intestinal wall, diagrammatic; *o*, ovary.

7. Transverse section; actual breadth 0.36 millimeter. *a*, vitellaria; *b*, intestine, diagrammatic; *c*, embryonic capsule containing ciliated twins; *d*, section of single young; *e*, probably section of ovum like that shown in fig. 1, *p*; *f*, mass of yolk cells; *o*, ovary. The intestine is on the dorsal side of the section.

8. Horizontal section in dorsal region; actual length 0.4 millimeter. *a*, vitellaria.

9. Epidermis separating from the body of a specimen crushed under cover glass in sea water, lateral view.

10. The same, vertical view.

11. Another lateral view of epidermal cells with basement membrane.

12. Epidermal cells showing degenerative changes. These changes took place rapidly under pressure.

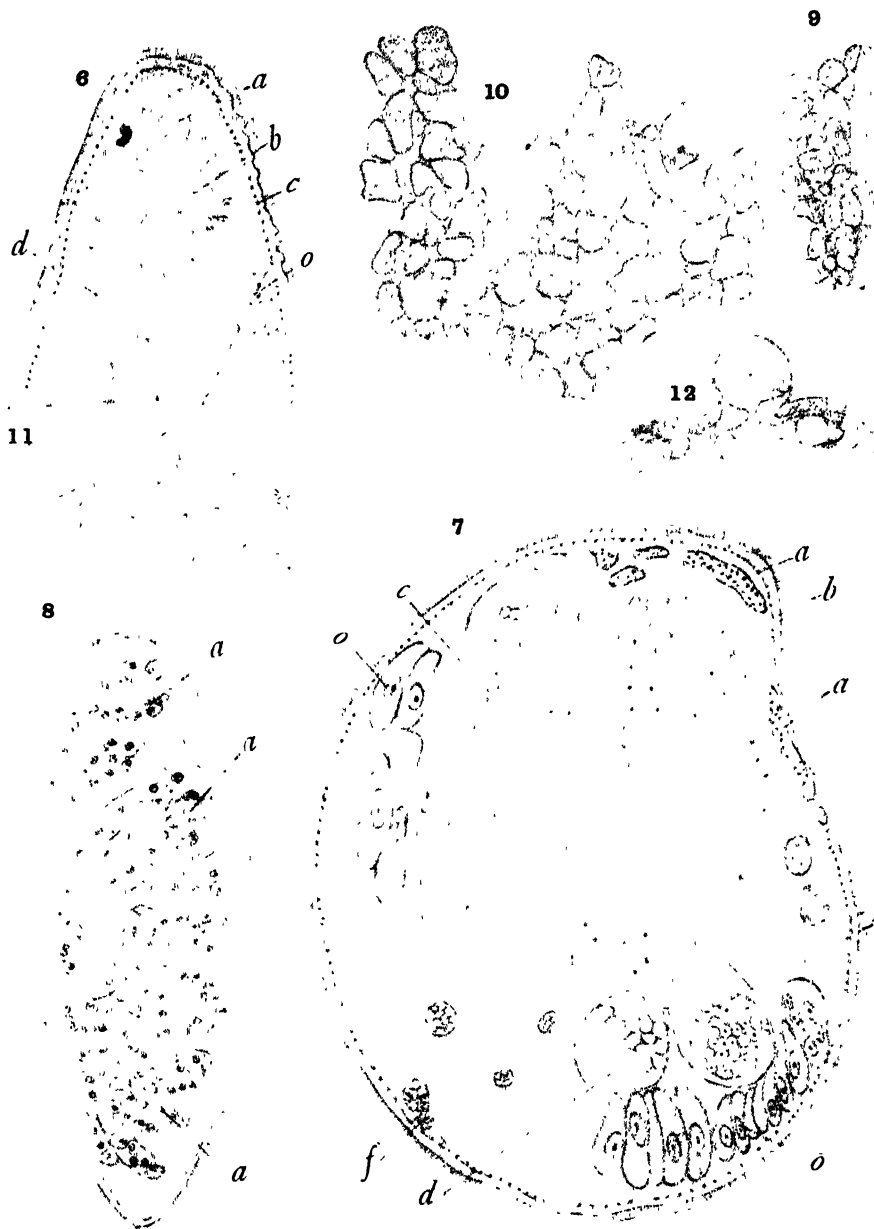


PLATE 3

EXPLANATION OF FIGURES

13. Nerve plexus, sketch from life of region posterior and lateral to eye.
14. Ganglion-like part of plexus much enlarged.
15. Plan of muscle fibers, sketched from section fixed in Flemming's fluid and stained with iron hematoxylin.
16. Collapsed embryonic capsule, life; length 0.07 millimeter.
17. Two of the same, from specimen mounted in balsam, length; 0.07 millimeter.
18. Another of the same from specimen mounted in balsam, length 0.05 millimeter.
19. Ovum from specimen mounted in balsam, longest diameter 0.09 millimeter.
20. Ovum with several cells in fine-granular yolk, same magnification as fig. 19.
21. Ovum with cluster of cells in midst of granular yolk.
22. Ovum with two clusters of cells at opposite poles separated by yolk.
23. Two ova, each with two embryos already formed in the midst of the yolk.
24. Three ova in place, representing different stages of development, from life.
25. Ovum containing only large nucleated cells, all the yolk has been absorbed; not a usual condition.
26. Twins in embryonic capsule, diameter about 0.04 millimeter.
27. The same, a little older than those shown in fig. 26.
28. Triplets in embryonic capsule.
29. Twins in capsule, eyes developed.
30. Another pair more highly magnified.
31. Sperm-sac, collapsed, free in mesenchyme, probably distorted by pressure.

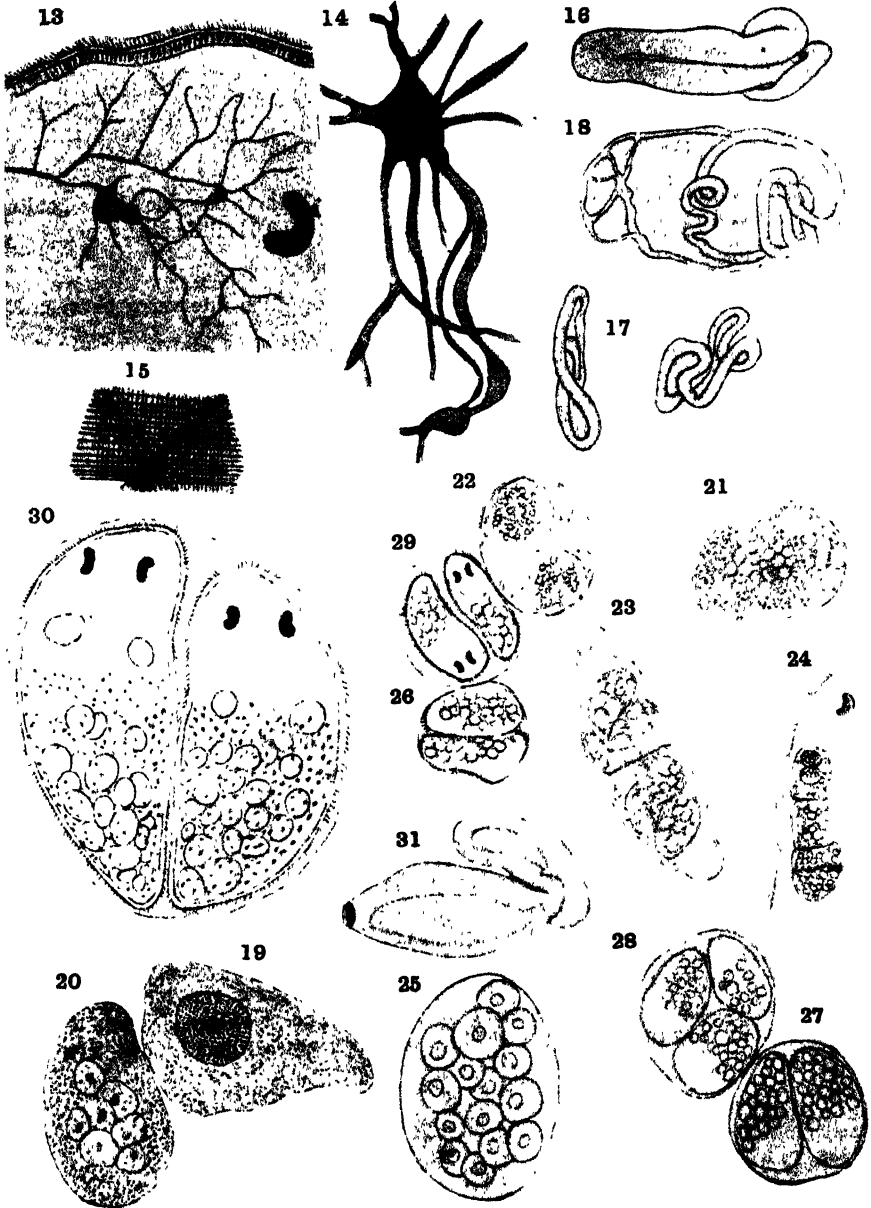


PLATE 4

EXPLANATION OF FIGURES

32. Tangential section through sperm-sac and a little to one side of the genital pore. *a*, uterus; *b*, sperm-sac; actual diameter of sac 0.05 millimeter.

33. Horizontal section. *a*, genital pore; *b*, uterus.

34. Horizontal section. *a*, penis; *b*, uterus; one section intervenes between 33 and 34.

35. Next section to 34. *a*, penis; *b*, uterus.

36. Next section to 35. *a*, sperm sac; *b*, uterus. Figs. 33-36 are drawn to the same scale; the diameter of the sperm sac in 36 is 0.05 millimeter. The sections were at least .005 millimeter thick.

37. Sperm sac, *a*, and ovum engaged in what appeared to be a kind of copulatory action; free hand sketch from life; *e*, wisp of spermatozoa (see p. 376 for description).

38. A somewhat similar condition is here indicated to that shown in 37; from specimen mounted in balsam. *a*, sperm-sac; *b*, ovum with two germ cells.

39. Empty sperm sac and ovum sketched from life; both were contracting somewhat rhythmically. *a*, sperm-sac; *b*, ovum.

40. Horizontal section showing the ovary-vitellarium. *a*, cells of ovary with the characteristically large germinal spots; *b*, yolk-forming cells; *c*, uterus; *d*, spermatozoa in duct; diameter of section 0.3 millimeter.

41. Another horizontal section, the fifth dorsal to the one shown in fig. 40. *a*, ovary; *b*, ovum with one cluster of cells showing in the section. A study of the succeeding sections shows that the ovum has another cluster of cells and is of the type shown in fig. 22. *c*, a nearly transverse section of a young worm. The section passes a little diagonally through the posterior end. Adjacent sections show that this is one of a pair of twins lying in their capsule. *d*, ciliated young with eye-spots. This specimen had escaped from its capsule. *e*, yolk-forming cells of the ovary-vitellarium; *f*, notch showing the continuation of the duct *c* in fig. 40, and continuous with the uterus of figs. 33-36. The positions of *b* and *d* were changed slightly to bring them within a smaller compass; breadth of section 0.3 millimeter.



STIMULI PRODUCED BY LIGHT AND BY CONTACT WITH SOLID WALLS AS FACTORS IN THE BEHAVIOR OF OPHIUROIDS¹

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THIRTEEN FIGURES.

The work of Preyer (1886-1887) on the reactions of echinoderms, in which he ascribed intelligence to these lowly creatures and saw in their behavior a true "will," has stimulated many to study the behavior of starfish, ophiuroids and sea-urchins. While all investigators since his time undoubtedly recognize the excellence of Preyer's observations and experiments, none have had the courage to endow echinoderms with intelligent action. Some, such as Romanes and Ewart (1881), deny the existence of any psychic quality in these animals; others, such as Loeb (1900), Glaser (1907) and Jennings (1907), who have repeated some of Preyer's experiments see no intelligence indicated, while such as von Uexküll (1905), and Bohn (1908), and probably some of the above-mentioned workers also, will neither deny nor affirm its existence, although their experiments do not lead them to believe that echinoderms possess intelligence. Observation and experiment up to the present time do not confirm Preyer's contention and we have no cases indicating associative memory among the echinoderms, yet Jennings (1907), Bohn (1908), and Cole (1910), have shown that starfishes form habits

¹ It gives me pleasure to thank the Carnegie Institution of Washington for the privilege of working in their marine biological laboratory. I also wish to express my appreciation of the courtesy and aid extended to me by Dr. Alfred G. Mayer, the director of the laboratory.

The beautiful coördination in the movements of the parts of the starfish has always been a subject which has occupied the minds of those who have observed the movements of echinoderms; we find nearly all who have studied the reactions of the starfish or sea-urchin offering observations, experiments and hypotheses to explain the harmonious working of the tube-feet. If one turns a starfish over so that it lies with its oral side uppermost for a longer or shorter time, the tube-feet wave about in an uncoördinated manner, but finally a time is reached when one sees a definite tendency for the tube-feet to move together seemingly in the interest of righting the animal. What is it that governs this sudden organized movement? To Preyer the phenomenon seems to be regulated by the nerve ring which acts as a centre; similarly, Romanes and Ewart recognized a coördinating centre as in part responsible for the action; von Uexküll, who seems to find no coördinating centre in the sea-urchin, considers coördination in the latter to be due to the action of one part on another; Loeb rules out the central nerve ring and also even ganglia as coördination centres, and judging from his work on the Medusae would probably consider the coördination as due to the simple facts of irritability and conductivity of the colloidal substances of the tissues, thus making the problem one of physical chemistry; to Jennings it is a physiological problem based on individual history and racial development; and finally to the vitalists the problem deals with a determining factor which is non-mechanical. We still seem to be as far from the solution as were Romanes, Ewart and Preyer.

During the last few years there has been much work done on the study of the reactions of the lower forms to photic, chemical, mechanical and electrical stimuli. Most investigators have paid especial attention and have laid especial stress on the uniformity in the response to a given stimulus. Jennings, however, both in his work on the lower organisms and also in his study of the starfish, has placed in the foreground the somewhat neglected idea that there is much variability in the reactions of organisms to a given stimulus; that differences in physiological states often cause differences in the reaction to this given stimulus. However, all who have studied the echinoderms have recognized the variabil-

ity in reaction. Preyer, Romanes, Ewart and von Uexküll speak of it, and Glaser looks upon the ophiuroid as "versatile." Bohn, whose paper on the reactions of starfishes and ophiuroids appeared shortly after that of Jennings on the starfish, recognizes variability in reaction and the influence of physiological states; he believes, however, and rightly, too, that certain external stimuli may be so strong as to annihilate any manifestations of internal states.

It is the purpose of this paper to record some observations made on ophiuroids under natural conditions and to describe some experiments undertaken in the laboratory to test the influence on movement, of light stimuli and stimuli produced by contact with solid walls. Incidentally, some observations and experiments are included dealing with the climbing of vertical walls and with the reactions to food. While many of the experiments made to test the effect of light and contact stimuli are of such a character as to produce considerable uniformity in the reaction to a given stimulus, and while it has been the writer's purpose to show the rather definite influence of these stimuli, yet every experiment and every observation has indicated that the behavior of the ophiuroid is not stereotyped. At times, when an ophiuroid is subjected to a great difference in light intensity, the reaction seems to the casual observer to be stereotyped; but this is not the case, for every now and then the behavior is such as to upset all calculations on the part of the observer. Given a certain definite external stimulus acting on an ophiuroid, one cannot always predict the resulting behavior correctly.

Several species of brittle-stars are quite abundant among the coral reefs and coral rocks in the region surrounding Loggerhead Key, Florida, and the clearness of the sea-water makes it easily possible to observe these animals in their natural habitat. The well-equipped marine laboratory of the Carnegie Institution, with its large aquaria and "live cars," affords the investigator an excellent opportunity to experiment with them.

There are seven or eight species of brittle-stars which are commonly found about Loggerhead Key. Of these, the large *Ophiocoma riisei* was used as a rule in the following experiments,

although *Ophiocoma echinata* and *Ophiura appressa* were frequently experimented with for comparison.

In order to keep the brittle-stars in the best condition possible for work in the laboratory, they were placed in large open "live cars," in which were pieces of coral and rock to afford them shelter. These "live cars" were floated in the open sea and were so arranged that there was a constant circulation of pure sea-water. Ophiuroids are extremely sensitive to impure water and they soon show the effects of the same, so care was taken when working with them to change the water frequently in order to prevent any unusual behavior.

LOCOMOTION

There seems to be a very general agreement, either definitely stated or implied, that in the case of ophiuroids no one ray has a greater functional value in locomotion than another, although, so far as I know, no careful statistical study has been made. Preyer (1886-1887), Grave (1900), Glaser (1907), Bohn (1908.)²

In other words, it seems probable that a normal ophiuroid may use any ray or interradius as a director, and apparently in the long run does not use one ray or interradius more than another. There are times, however, during experiments and even under natural conditions when an ophiuroid may use a certain ray or interradius as director exclusively. This behavior can usually be traced to the lasting effect of some previous stimulus, such as contact with a more or less vertical surface or to some stimulus such as difference in light intensity.

TUBE-FEET AS LOCOMOTOR ORGANS

The statement that the ambulacral appendages of the ophiuroids do not act as locomotor organs can no longer be accepted since the studies of Grave (1900) on *Ophiura brevispina* and von Hj. Östergren (1904) on *Ophiocoma nigra*.

² Bohn, however, finds that sometimes large specimens of the starfish, *Asterias rubens*, show "une sorte de préférence pour certain bras" (p. 29); Cole (1910), as a result of his statistical study of the locomotion of the starfish, finds that there is a tendency to use certain rays more than others.

My observations on the ophiuroids found among the coral reefs near Loggerhead Key confirm the observations of these two investigators and show that these appendages are important both in climbing and feeding. The ambulacral appendages of *Ophiocoma riisei* seem to be quite well developed when compared with those of some other ophiuroids, although they are not so serviceable in locomotion as those of the starfish. Grave (1900) has found that *Ophiura brevispina* uses the tube-feet in ordinary locomotion by fitting them into the irregularities of the surface, thus forming hold-fasts for the rest of the arm to pull against. The same behavior was observed in *Ophiocoma riisei*, but as in the case of *Ophiura brevispina*, when an individual moves fast it does so by the stroke movement of the rays and the ambulacral appendages do not seem to aid. Jennings (1907) has shown that in the starfish, *Asterias forreri* de Loriol, the appendages are used in a somewhat similar manner to the human leg. This is probably true for all starfishes, but only to a small extent for the ophiuroids. When an individual moves slowly such movements of the tube-feet may be seen but they are not very important in locomotion.

Until the publication of the observations of von Hj. Östergren (1904) on the climbing of *Ophiocoma nigra* it was generally supposed that the brittle-stars did not use their tube-feet in a sucker-like manner. My study of *Ophiocoma riisei* shows that while this form has no definite suckers, such as we find at the end of the tube-feet of starfishes and sea-urchins, yet its tube-feet have an adhesive function which is brought into play principally while the creatures are climbing up rocks or other vertical surfaces. If a specimen of *Ophiocoma riisei* is placed with its oral surface against the side of a glass aquarium filled with sea-water and held there for a few seconds until the tube-feet can be applied to the glass the animal will fasten itself and remain for some time. Close examination with a lens will show that many of the ambulacral appendages have their tips closely pressed against the glass, and that often when they are drawn away they loosen their hold with a little jerk, showing that something was holding them. No adhesive matter could be found at the ends of the tube-feet nor

could any mucous glands be seen in their tissues. While there are no definite suckers present, it is undoubtedly true that a temporary sucker is formed when the foot presses itself against the glass.

It is not only when the ophiuroid is held against the side of the aquarium that it adheres, for it often does so of its own accord, climbing up almost to the surface of the water. This occurs usually when there are no rocks or crevices for shelter at the bottom of the aquarium. I have observed similar behavior in the case of *Ophiocoma echinata*. *Ophiura appressa* will also adhere if it is held for a time against the glass but neither of these two species is so well adapted in this direction as *Ophiocoma riisei*.

It might be claimed that the ophiuroids which climb the sides of the aquarium are "positively phototactic" or "positively photopathic," but this can hardly be true because the ophiuroids are well known for their tendency to move into places where the light is very dim. We might also say that they are "negatively geotropic;" but if we mean by this that they are compelled to move up the sides of the aquarium on account of the stimulus produced by the attraction of gravitation, I do not believe that we have used the correct term. On the contrary, I believe that *Ophiocoma riisei* as the result of certain stimuli such as difference in light intensity or unfavorable conditions either external or internal, forms the impulse to move in a certain direction; having reached the wall of the aquarium it will continue moving forward and in so doing mount the wall unless the conditions of contact alter the impulse when it may crawl along the base of the wall.

TUBE-FEET AS FEEDING ORGANS

Ophiocoma riisei sometimes takes its food as von Uexküll (1904) describes; that is, by a twisting of the arm which progresses from the point at which the food particle touches the tube-feet to the region of the mouth, but in most cases the food particle (*e.g.*, piece of fresh fish) is taken by the tube-feet and carried along by them until it reaches the mouth.

Hamann (1900) and Cuenot (1891) state that the tube-feet are taste organs or organs of smell, while Preyer (1886-1887) and Nagel (1894) believe the tentacles or tube-feet in the region of the mouth have the same function.

While watching the feeding of *Ophiocoma* I became interested in testing the tube-feet in this respect. In order to see how the ophiuroid reacted to what was food and what was not, a small pellet of paper was used instead of the particle of fish. This was placed close to a ray and, as a rule, it was picked up by the tube-feet, carried along for about two or three centimeters and then dropped. When a small piece of fish was substituted for the pellet of paper it was as a rule carried the full length of the ray to the mouth. This seems to show that the ophiuroid reacted to food without bringing it to the mouth although of course the evidence is not conclusive.

Several series of tests were made with rays amputated about one centimeter from the disc and placed with their oral *i.e.*, tube-feet surface uppermost. A small piece of fish placed close to the tip of the ray was taken up at once by the tube-feet and carried in a quite normal manner to the cut end of the ray where it was dropped. When, however, a small pellet of wet paper was used, although it was taken up at once, it was not carried to the proximal end of the ray, but was simply rolled about through a distance of a centimeter or two and then dropped.

It would occur to anyone that in the test just described we might be dealing with the tactile sense, and that the consistency of the fish and paper might have something to do with the result, so the following test was made. Two pellets of wet paper of approximately the same size and consistency were prepared. One of these was dipped in sea-water in which a piece of fresh fish had been soaking and the other was merely dipped in sea-water. A ray which had been amputated an hour previously was then tested with these two pellets, as in the above experiment. The pellet without fish flavor was taken up by the tube-feet and almost immediately rejected, while the one with the fish juices on it was seized and carried rapidly to the cut end of the ray. A repetition of these tests at intervals of five or ten minutes gave the same results as those just described.

These experiments undoubtedly show that the rays react to food even as far out as the tip and independently of the mouth or the rest of the disc. Furthermore, they indicate that sense organs are present either on the tube-feet or on the ambulacral surface of the rays, which are sensitive to organic substances in solution or suspension and that some of these substances stimulate the tube-feet either directly or indirectly in such a manner that objects are carried toward the mouth, while others do not.

METHOD OF RIGHTING

The method of righting of ophiuroids has been studied by Grave (1900), von Uexküll (1904) and Glaser (1907). Grave (p. 87) says: "Two adjacent arms straighten out so that together they form a straight line. On these arms as an axis the body revolves, being pushed over by the three remaining arms, but mostly by the median one of the three." Von Uexküll (p. 11) finds that when *Ophioglypha* is placed on its aboral surface, the rays become bent under so as to raise the disc off the bottom and the weight of the disc causes the ophiuroid to topple over into its normal position. Glaser (p. 208) recognizes a factor in the mechanics of turning which I think is quite important. He says that movements occur at the bases of the straightened arms and in the inter-radial portion of the disc between them whose effect is to start the righting.

In fresh, healthy specimens of *Ophiocoma echinata*, which have not been tired out by experiments and which have been kept in pure sea-water, the behavior in righting is as follows: As soon as the specimen is placed on its aboral side the tube-feet begin to move in all directions, and almost at the same time the disc is raised off the bottom by the stiffening and pushing of the rays. Then two of the rays spread out much as an acrobat "doing the split," their distal ends twist over into their normal position and the disc begins to turn over. Usually the leverage seems to be at the proximal end of the rays near where they are attached to the disc (i.e., the two rays forming "the split"). There seems to be a decided twisting as a result of the contraction of certain

muscles of the rays which are attached to the somewhat rigid skeleton. This twisting starts the turning over of the disc which, after a certain point, continues as the result of its own weight, drawing the other rays over after it. The tube-feet do not aid in the righting nor do the three rays which are drawn over help as a usual thing. In fact, often very active specimens seem to right themselves without raising up the disc but simply by twisting the proximal parts of the two rays forming "the split." It must be remembered that *Ophiocoma echinata* and *Ophiocoma riisei* have comparatively long and heavy rays.

In order to test the assumption that the twisting of the rays is an important point in righting, three of the rays were amputated close to the disc of a specimen which was then placed in a dish of sea-water with the ambulacral surface uppermost. Almost immediately the two remaining rays assumed "the split" like position, the distal ends turned over and the twisting continued until the disk was brought into its normal position. This series of movements was accomplished with apparently little difficulty and the specimen was even successful in righting itself when some pressure was applied to the edge of the inverted disc from which the rays were amputated.

Four rays were removed from another individual, which was then inverted and in a similar manner by the twisting of a single ray the disc was righted.

These experiments seem to show quite clearly that the twisting of the rays is an important part of the righting of *Ophiocoma echinata* and that the righting can be accomplished by the movements of the two of the rays without the aid of the other three.

BEHAVIOR UNDER NATURAL CONDITIONS

Before discussing the experiments carried on in the laboratory to test the behavior toward light and other factors, I shall describe some observations made on ophiuroids in their natural habitat. One of the best places to find these creatures, in the region around Loggerhead Key, is on the western side of a typical coral reef lying a short distance off the east side of Bird Key.

Part of this reef is exposed at low tide and part is not. Under the light rocks of the unexposed part many ophiuroids live. If one of these rocks is turned over, in the majority of cases, several specimens are brought to light, and among these one usually finds *Ophiocoma riisei* and *Ophiura appressa*.

It is well known that ophiuroids are sensitive to light. Some of them (*Ophiocoma riisei*) seem to be very sensitive, while others, such as *Ophiolepis ciliata*, which Bohn (1908) studied, are not so sensitive. In general, however, I think it may be said that ophiuroids react negatively to brightly lighted fields unless some other factor changes the reaction. Now let us see what occurs when a rock, covering ophiuroids, is overturned on a bright day. As a usual thing it will be found that they move in the direction toward which the stone has been turned without reference to the position of the sun. This does not mean that they are not sensitive to brightly lighted regions but that there are some other factors which determine the direction of movement. The ophiuroid usually has one or more rays touching the under surface of the rock or twisted around some projection, and the stimulation produced by this contact is retained by these rays so that they move forward with these rays in advance in the direction the stone has been moved. In other words, they "show memory of past stimulation" as Jennings (1907) calls it. The stimulus of contact with some raised solid surface, such as the side of a rock, is retained for some little time as we shall see in experiments to be described later. Even a small stone lying on the bottom often has an effect on a moving ophiuroid when a ray touches it; the contact is very apt to cause hesitancy when the creature is moving in a certain direction even though the stone affords practically no shadow and no shelter.

The following behavior also shows the importance of contact stimuli in the movements of ophiuroids. If a specimen which is lying under the shelter of a rock with one or more rays against it is drawn out with the hand, keeping the rays in the same general position with reference to the shelter, the ophiuroid will usually return to about the same place if it is not removed to too great a distance. This behavior is apparently not a reaction to the shade

of the rock, for if the specimen is turned around while being taken away from the rock it will usually move with the ray or rays directed forward, which were originally against the rock, even though this behavior may take the ophiuroid directly away from the shelter. The influence of handling by the investigator at once suggests itself as a factor in the behavior just described. In fact, experiments below show that echinoderms do tend to move away from the side of the body which has been handled, but the explanation of the behavior given above nevertheless holds good, as is shown by changing the manner of holding.

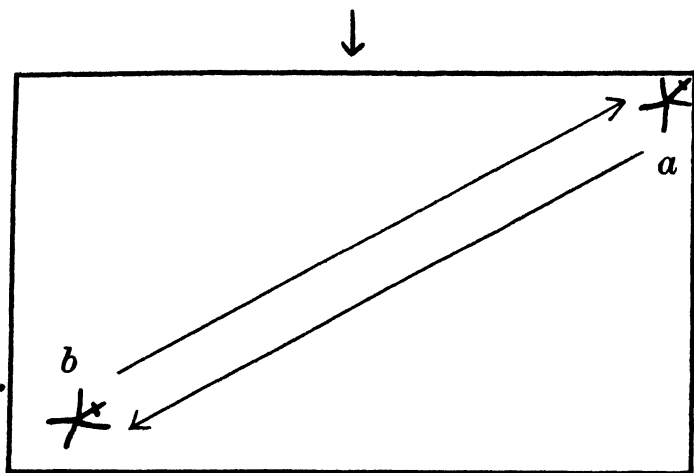
CONTACT WITH SOLID WALLS

It has been mentioned above that stimuli produced by contact with solid walls, such as the surface of rocks, are very important factors in the life of the ophiuroid. Experiments which will be described below indicate that they are even more important than stimuli produced by small differences in intensity of light.

The following rather crude experiment shows the lasting effect of a stimulus produced by contact with the solid walls of the corner of a rectangular glass dish. This dish, 36 cm. long, 23.5 cm. wide and 7.5 cm. high, was partly filled with sea-water and placed in front of a wide-open window. Light from many sources entered the dish but that from the open window was of the greatest intensity. An ophiuroid (*Ophiocoma riisei*) was placed in the dish and it moved at once to the corner indicated, (Fig. 1, *a*) placing one of its rays in the angle. After allowing the specimen to remain there for several minutes, it was pushed diagonally across the dish to the position shown (fig. 1, *b*). The orientation of the ophiuroid with reference to the original resting place was not changed during this procedure and its rays were not allowed to touch the sides or corners of the dish. Almost immediately the creature moved back to the corner from which it had been removed, (Fig. 1, *a*) even though in so doing it went toward the strongest source of light. It did not turn the disc but moved with the ray forward that had originally been in contact with the corner. This experiment was repeated many times, using different corners for the starting point, and in nearly every case the ophiur-

oid returned to the corner in which it had been resting, although sometimes, especially when fatigued, the return was not so direct as that shown in fig. 1.

One naturally asks if the behavior just described is due to the persistence of the contact-stimulus? May not bilateral symmetry,



EXPLANATION OF FIGURES

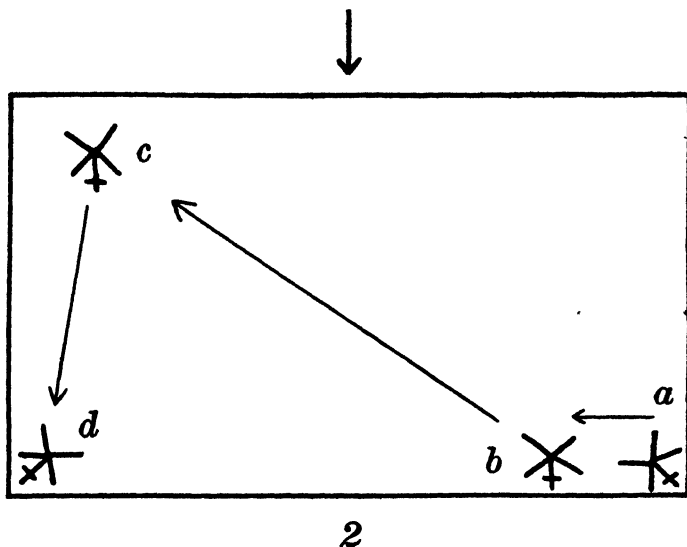
The large rectangle in the thirteen figures of this article represents the outline of the large rectangular glass dish; the arrow outside of the rectangle indicates the direction from which the most intense light comes; the plain arrows inside the rectangle show the direction of locomotion; the arrows with hooks on the end indicate that the ophiuroid has been turned on its back or that it has righted itself; the ophiuroid is represented by a five armed star and in any one figure it is always the same arm or ray that is marked by a short cross line.

method of handling, intensity of the lighted field, or currents in the water, due to pushing the ophiuroid away from its resting place be factors?

In order to see if bilateral symmetry or any structural peculiarity were factors in the above behavior, many trials were made in

which each different ray was subjected to the stimulus produced by the corner contact. There is no bilateral symmetry evident on the surface of *O. riisei*, so track was kept of the different rays used simply by watching them closely and also by marking the rays with a small loop of thread. The results showed conclusively that no one ray had any greater functional value than another.

While there is no doubt that the method of taking hold of an ophiuroid may have an effect on its behavior, yet by varying this



method it was found that the behavior under consideration was not due to the handling.

Further to test the persistence of this contact stimulus, the following two experiments were tried. A specimen with a ray resting in the corner (fig. 2 *a*) was turned so that this ray was directed as shown at *b* and then pushed to the position *c*. This specimen then instead of returning to position *a* or *b* moved to the position *d*. That is, it moved with the ray forward that had originally been in contact with the corner at *a*.

In another experiment an individual resting at *a* (fig. 3) was turned through 180° (*b*), then pushed to position *c*. It then moved

directly to the corner *d*, with the ray leading that had been in the corner at *a*.

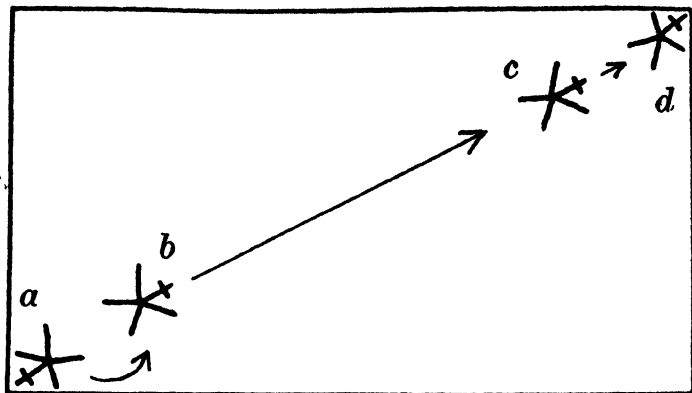
These two experiments show again in quite a clear manner the persistence of the stimulus produced by the contact or breaking of the contact of the ray with the corner of the dish.

The current in the water produced by pushing the ophiuroid from one region to another seems to have no effect on the behavior. If, for example, we pick up the ophiuroid and carry it out of the water to another position in the dish the behavior is the same as when the specimen is pushed through the water. An experiment was tried in which an ophiuroid was taken from the corner in which it was resting (fig. 4, *a*), and was then carried around the room as indicated. It was placed at *b* and immediately began to move with the marked ray as director toward its original resting place, but finally turned and stopped in the corner *c*. The behavior here was not quite so characteristic as usual, but the tendency to use the ray that had been stimulated was evident. Similar trials in which the test was less complicated gave much more accurate results.

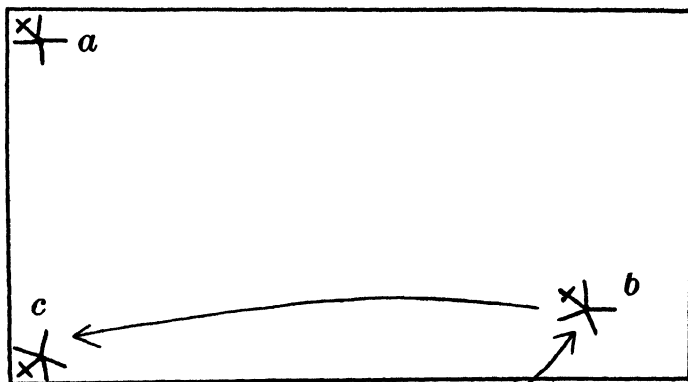
I have described only a few experiments of over a hundred that were tried, and I do not wish to leave the impression that the results were always as definite as those mentioned or that there were no other factors operating during the experiments; but one cannot avoid the conclusion that the stimulus produced by the contact or breaking of contact of the ray with a solid wall is a very important factor in the behavior of the ophiuroid. The effect of this stimulus often persists for some little time, but it may easily disappear and in fact be completely dominated by large differences in the intensity of light.

CONTACT STIMULI AND LIGHT STIMULI

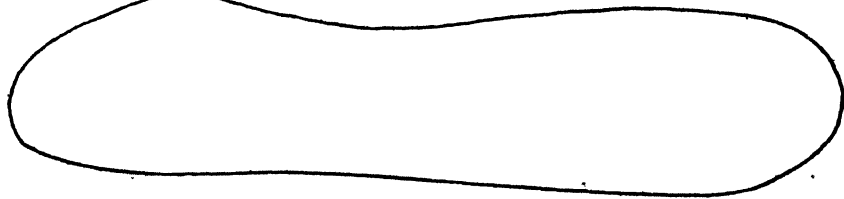
In order to compare the effects of contact stimuli due to solid walls and the stimuli due to dimly lighted regions, many series of experiments were tried. Their relative value in determining the direction of locomotion was tested.



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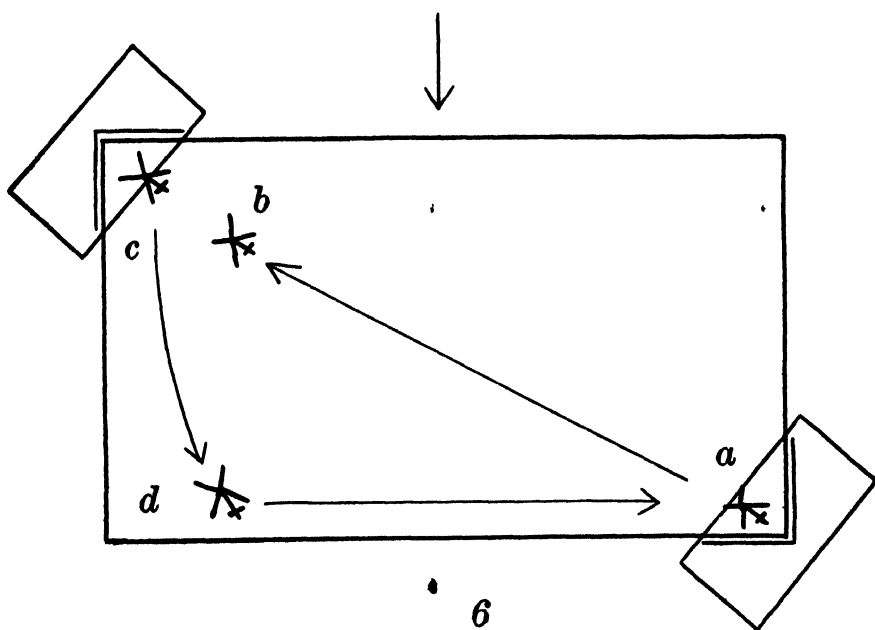
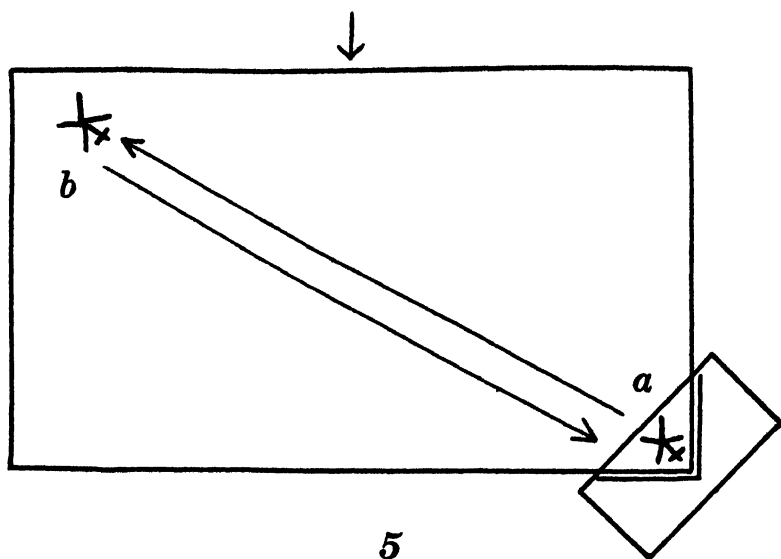
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We shall consider only one of these series which is a characteristic one. In the first place an ophiuroid was submitted to several trials such as have been described above and it was found that the behavior was as usual. That is, the contact stimulus in the corner of the glass dish persisted as a rule and seemed to be the determining factor in the specimen's return when, without changing its orientation, it was pushed away. Then the corner in which the ophiuroid was resting was shaded by placing dark screens around it. As one would naturally expect, since it is known that ophiuroids react positively to dimly lighted regions, the specimen moved in the usual manner when put to trial (fig. 5). That is, after being drawn out from the darkened corner, without change of orientation, it returned again, with the contact ray directed forward. The behavior on reaching its resting place was somewhat different from that exhibited in the preceding experiments. The ophiuroid assumed a more settled attitude; it curled its rays up to some extent, thus occupying a smaller space.

After several trials the conditions were changed (fig. 6) by also placing dark screens around the corner, diagonally opposite to the one originally shaded. The ophiuroid was then pushed to the position *b*. After some hesitation, undoubtedly due to the new shaded region, it moved to *c*, but without touching the corner walls, went to *d* and finally back to *a*, assuming its original attitude with the same ray in the corner. The behavior seemed to indicate a conflict between two guiding stimuli; the shaded region apparently had an influence, for the specimen changed its usual direction of movement and went into the shaded pocket *c*, using the interradius opposite to the contact ray as director. Almost immediately, however, it came out again and moved by the way of *d* to its original resting place with the contact ray leading, apparently still under the influence of the contact stimulus received at *a*.

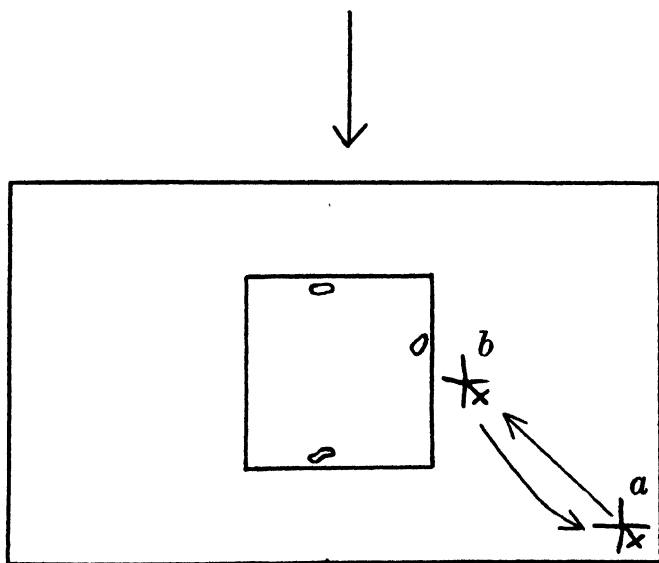
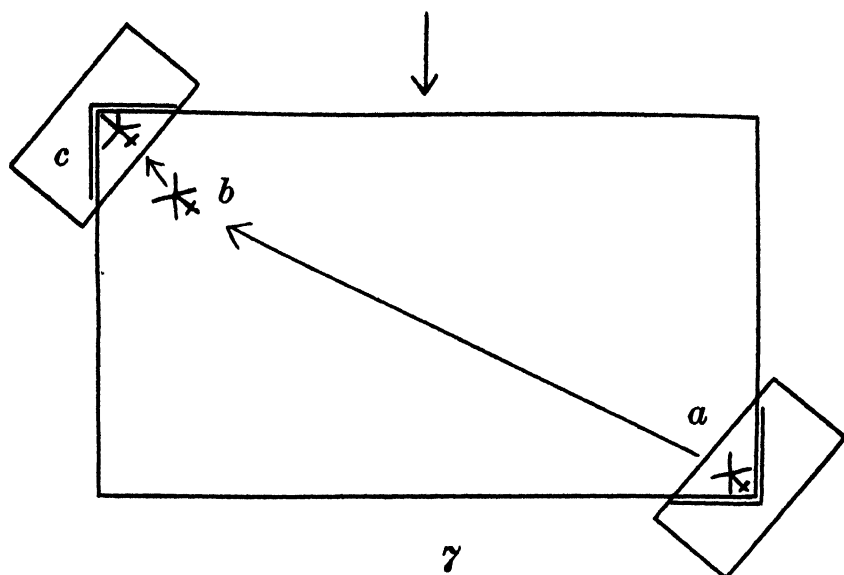
The experiment just described was repeated seven times, first using one shaded corner as the starting point and then the other shaded corner. In every case but one the behavior was the same, except that the specimen remained in the shaded corner into which it had moved (fig. 7). The ophiuroid moved to the shaded



region opposite to its original resting place; that is, apparently without reference to the contact stimulus. In other words, the stimulus produced by the proximity of a shaded region seemed to dominate over the stimulus produced by contact with the corner walls.

The above series of experiments was finally completed by submitting the ophiuroid to a number of trials similar to those described at the beginning of the series. That is, the screens were removed from both corners and the specimen was tested in respect to the effect of the contact stimulus. Its behavior now became similar to that at the beginning of the series. The ophiuroid reacted definitely to the stimulus produced by contact or breaking of contact with the corner walls of the dish. It must be mentioned, however, that the return to the starting point was not so accurate, a fact which may be due to a persisting effect of the stimulus of the darkened regions or to fatigue.

From these experiments we may draw the following conclusions: Solid walls and darkened regions are important factors in the behavior of *Ophiocoma riisei*, *Ophiocoma echinata* and *Ophiura appressa*. The stimulus produced by the contact or breaking of contact with solid walls, especially a corner, persists for an appreciable time, long enough so that the ophiuroid will react to the stimulus some little time after and will tend to return to the object producing it, or more accurately, will tend to move with the ray or rays that received the stimulus forward. A region of reduced light intensity, such as the dark pocket in the above experiments, also acts as a stimulus on these ophiuroids and produces a positive reaction. The darkened region seems to have an effect from some little distance (several centimeters) a phenomenon observed by Jennings (1907) for the starfish and by Bohn (1908) while testing the effect of light and dark screens on ophiuroids. This phenomenon will be discussed later. An analysis of the relative value of these two factors in the behavior seems to show that the stimulus produced by a region of much reduced light intensity is more important than the contact stimulus in determining the direction of locomotion. However, when these ophiuroids move into a darkened region the subsequent behavior is dependent on



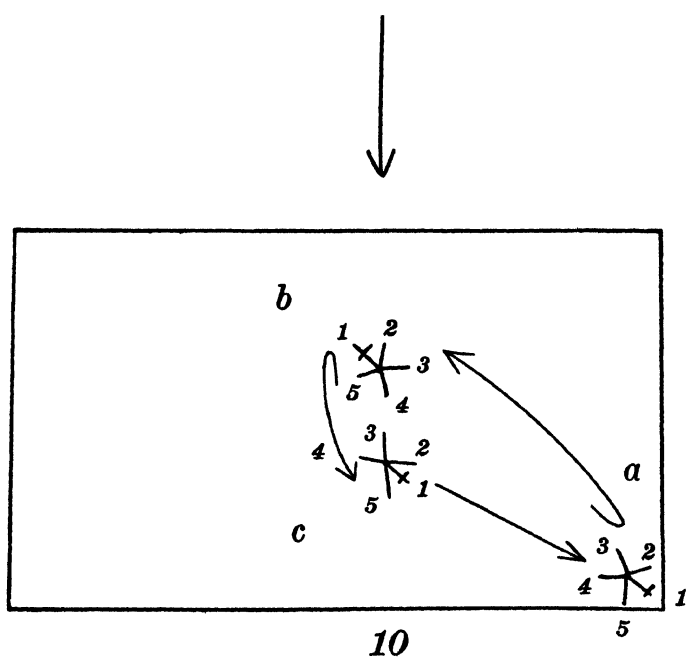
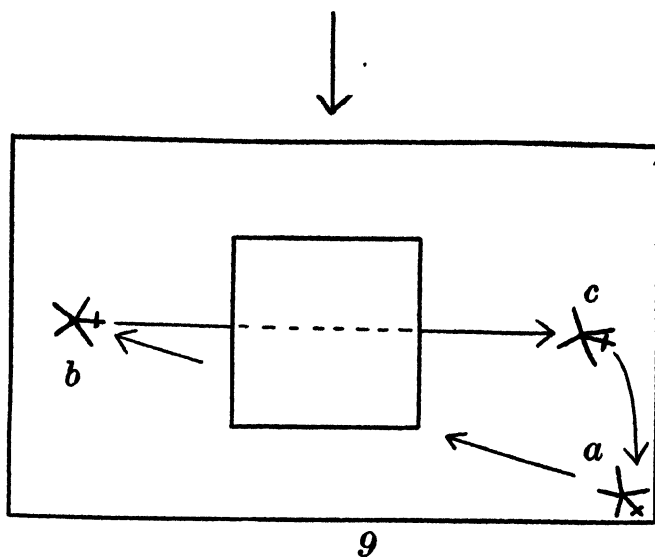
whether there are solid objects such as stones or walls, against which they may put their rays. That is, they will not, as a rule, remain in the darkened region unless such objects are present.

EFFECT OF HORIZONTAL DARK SCREENS

In order to determine the effect of darkened regions with or without the stimuli produced by contact with solid walls, a series of experiments were made, in which a region of shade was produced in the middle of the experimenting dish by supporting a piece of black glass in the water horizontally by means of three pieces of coral (fig. 8). In this way a shadow was cast on the bottom of the dish without forming a pocket, such as that in the experiments described above. A region of reduced light intensity was produced in the field without introducing any dark vertical walls.

Many trials were made similar to that shown in fig. 8, in which the ophiuroid was removed from its resting place *a* and pushed to the position *b*, close to the shaded region. In no case did the specimen pass under the screen, the behavior thus differing from the experiments described above, in which there was a dark pocket. This difference may have been due to the greater light intensity of the field in the former case than in the latter, but I believe that the important factor was the absence of vertical dark walls, which seem to have an effect, as Jennings (1907) and Bohn (1908) have shown.

After the series of trials just described, another series was tried, in which the black screen was suspended in the water in a horizontal position about two centimeters from the bottom without using any pieces of coral for support. Again and again an ophiuroid was tested in the manner shown in fig. 8, and also in fig. 9. In the former case the specimen did not move under the shaded region, and in the latter, although it passed under it, it never remained, but returned to the original brightly-lighted resting place, unless by chance one of its rays touched the hanging screen, when it stayed under, although its rays did not remain in contact



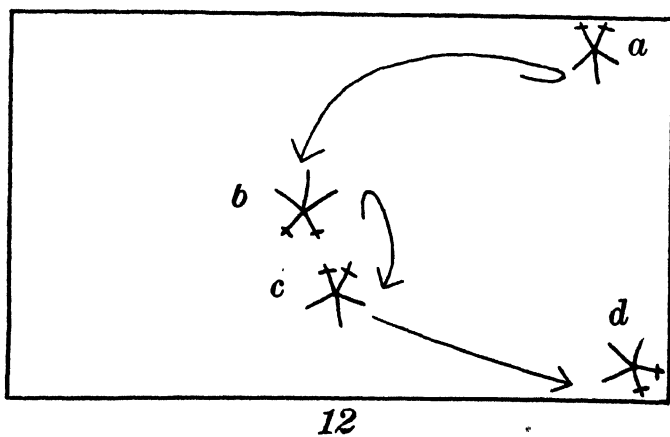
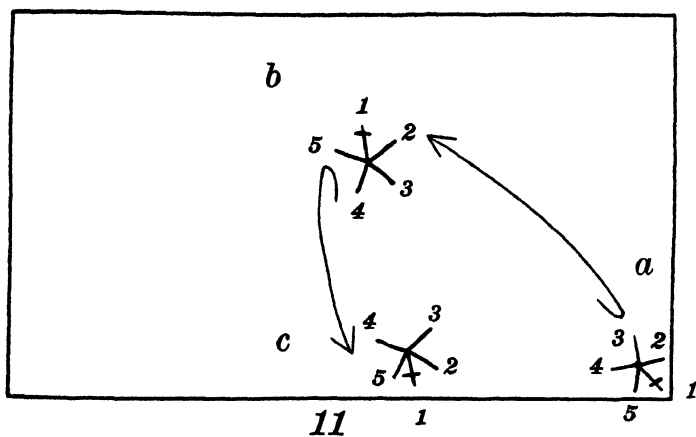
with the latter. If a piece of coral was placed under the horizontal screen the behavior of the ophiuroid was almost invariably changed.

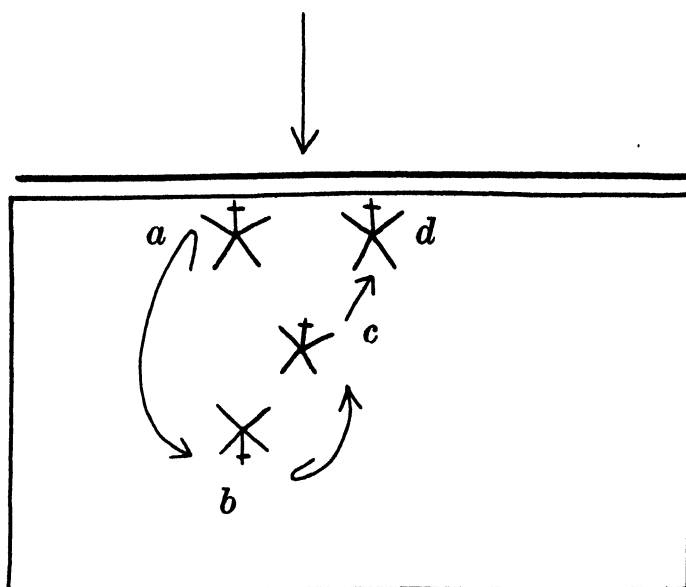
Instead of moving through the shaded region, the specimen put one or more of its rays against the coral, tilted its disc and remained quiet as though the conditions for its comfort were satisfied.

EFFECT OF LIGHT AND CONTACT STIMULI ON DIRECTION OF RIGHTING

The ophiuroids with which I have worked do not seem to show any permanent habit of using a certain pair of rays on which to right themselves. That is, when one of the creatures with rays of about equal length is inverted in a dish of water, care being taken to rule out light, handling, etc., no one pair seems to have greater functional value than another. Normal structural characters do not seem to determine the rays used, but when such external factors as light, contact with solid walls and handling are allowed to operate, they seem to be of much importance in determining the direction of righting and consequently the rays used. Undoubtedly internal physiological conditions do at times influence the behavior, but there does not seem to be a permanent habit of righting on a certain pair of rays persisting from day to day.

In the series of experiments from which the following typical cases are taken, the same glass dish filled with sea-water was placed in front of a wide window as in the previous experiment, so that the strongest light came from the side indicated by the straight arrow in the figures. An ophiuroid was placed in the water and allowed to come to rest with one of its rays in the corner *a*. The rays were then numbered, as shown in fig. 10, ray 1 being the ray in contact with the corner. When the ophiuroid was taken from the position *a*, inverted and placed at the position *b*, so that the ray 1 was pointed in a diametrically opposite direction, the creature righted itself on rays 3 and 4, returning directly to its original position *a*. Similar trials were made many times, and as a general rule the behavior was the same as that just described. Now the





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question arises: Are we dealing here with the effect of light stimuli or with the effect of stimuli produced by contact with the wall of the dish or with both?

The following experiment among many others shows that the stimulus due to contact is to be reckoned with; in other words, that the stimulus produced by the contact of ray 1 with the solid walls of the corner of the dish or the stimulus produced by the breaking of this contact has an effect on the direction of righting, on the rays employed in this righting, and on the direction of locomotion directly after the completion of the act. An ophiuroid (fig. 11) resting at *a* against the corner walls was inverted and placed at *b*, so that ray 1 was not directed in a diametrically opposite direction to that in position *a*. The result was that the ophiuroid righted itself on rays 3 and 4, and then moved, with ray 1 as the directing ray, apparently under the influence of the stimulus given to ray 1 when it was resting in the corner *a*. For

this reason, it would, seem the specimen did not return to its original resting place.

While the contact stimulus received before the ophiuroid is inverted surely is a factor in determining the direction of righting, the intensity of the light is undoubtedly a more important one. It certainly plays an important part in the two experiments just described. Both factors are responsible for the behavior observed, for when similar trials were made, in which the stimulus of contact with the corner walls was such as would tend to make an ophiuroid right itself toward the bright light of the window, the creature did not, as a rule, right itself in that direction. Such a result is shown in fig. 12. An ophiuroid at rest in the position *a*, with two rays against the wall of the dish nearest the window was inverted and placed at *b*. Instead of righting itself toward the window as would be expected if the stimulus received from the contact with the wall were operating, it righting away from the window, using the two rays marked and then moved to the position *d*.

When, however, as is shown in fig. 13, the side toward the window is darkened, with an opaque screen an ophiuroid resting at *a*, when inverted and placed at *b*, will right itself toward the darkened side.

DIRECTION OF LOCOMOTION WITH INTENSE LIGHT COMING FROM ONE DIRECTION

In all the experiments described above no especial attention was paid to the lighting. The ophiuroids were exposed to light rays coming from many directions, and although the experimenting dish was placed in front of a window, the specimens were not subjected to great differences in light intensity. As a result, the reaction to light was not nearly so pronounced as in tests made in a box painted dead black inside and open only at one end. Such a box was placed with the open end directed toward the very intense light reflected from a bank of white sand; inside of it was put a rectangular dish, lined with dead black paper and filled with sea-water. Under such conditions when an ophiuroid was placed

in the dish it almost immediately and almost invariably moved to the darkened end of the dish. Previous stimuli produced by contact with solid walls and methods of handling seemed to have no effect. The experiment was repeated many times, varying the position of the rays with reference to the open end of the box, and almost always the reaction was negative to the light. Usually after a great many trials the ophiuroids showed signs of fatigue, and then might fail to react or even might move in a positive direction. However, the tests showed plainly that the ophiuroids experimented with as a rule react strongly in a negative manner to intense light.

RIGHTING WHEN EXPOSED TO INTENSE LIGHT FROM ONE DIRECTION

When a normal *Ophiocoma riisei* is placed with its oral side uppermost in the apparatus just described, it rights itself immediately and quickly. A series of trials made under these conditions showed that the ophiuroids righted almost invariably away from the light. The position of the rays was varied in these tests and also the manner of handling, but the behavior in almost every case resulted in a righting toward the darker end of the dish. While the righting was not always directly away from the lighted end, yet it was evident that the impulse was to right away from the light. After the specimen had regained its normal attitude with the oral side down, it moved almost invariably to the dark end of the dish.

It is of considerable interest to compare the behavior of this ophiuroid with that of the starfish, *Echinaster crassispina*. We have seen that the ophiuroid reacts negatively to bright light; that is, it rights itself away from the source of the light and then also, after regaining its normal position, continues to move away from the source. On the other hand, *Echinaster crassispina*, which reacts positively to bright light when moving under normal conditions, rights itself in the same manner as the ophiuroid, *i.e.*, away from the light. So we see the ophiuroid on being inverted rights itself away from the light and then continues to move away

from the light, while the starfish *Echinaster crassispina* under similar conditions, also rights itself away from the light, but then moves towards the light. In other words, the intense daylight coming from one direction produces a negative reaction when it strikes the aboral surface of the ophiuroid and also a negative reaction when it shines on the oral surface; on the other hand, this same intensity of light brings about a positive reaction when it stimulates the aboral surface of the starfish, *Echinaster*, and a negative reaction when it stimulates the oral surface. It appears then that the same intensity of light acting on the oral and aboral surfaces of *Echinaster* brings about reactions of opposite signs.

INFLUENCE OF VERTICAL DARK AND LIGHT WALLS

All of the most recent workers in the field of light reactions have recognized the importance of the influence of the reflection of light rays from various surfaces in the region of the organism experimented upon. Bohn in some of his earlier papers and in his paper on the reaction of starfish (1908), and also Mast (1907) and Cole (1907), in their very careful work, have laid special stress on the necessity of taking this factor into account. Both Jennings (1907) and Bohn (1908) find that the direction of movement of a starfish, which ordinarily reacts negatively to bright light, is influenced by vertical dark walls even when they cast no shadow; that is, these starfish when not too far away from the dark wall will react by moving toward it. Bohn also shows that other starfish which react positively to bright light move toward a light colored wall rather than a dark one. Another point on which Bohn (1908) lays stress is that shadows cast on the horizontal surface upon which the starfish lies have but little effect in determining the behavior of the creatures. While at present I can offer no quantitative proof of the correctness of this view for ophiuroids, yet my experiments with hanging dark screens indicate that it holds good.

EFFECT OF HANDLING ON THE METHOD OF RIGHTING

It is generally believed that taking hold of the ray of a starfish or an ophiuroid has an effect on its subsequent behavior. I thought it worth while to make a series of tests with ophiuroids to determine the effect of handling on the method of righting. The apparatus used for these trials was a tight, deep, wooden bucket, into which no light entered except from above, and even this light was reduced to a minimum by partial covering. Several specimens of *Ophiocoma riisei* were tested. An ophiuroid was picked up by one ray, inverted and placed at the bottom of the bucket, which of course contained sea-water. The rays on which the ophiuroid righted itself were noted and then the specimen was allowed to rest for several minutes. Taking hold of the same ray the experiment was repeated five times; then another series of five trials were made, taking hold of another ray. In this way twenty-five trials were made taking hold of a different ray after every five trials. Although the light coming in from above probably was not a determining factor in the experiment, yet in order to make sure it had no influence, the position of the ophiuroid was varied when inverted, so that the handled ray was directed in a different direction during each of the five trials, the whole five covering the circumference of a circle.

The results of these trials were very conclusive. Almost invariably the ray handled was not used in the righting, although sometimes an adjacent ray took part in the reaction. The tendency to right away from the stimulated ray was very evident. However, if unhealthy ophiuroids are used, or if fatigue is produced by too many trials closely following one another, the results may be different. Similar tests made with the starfish *Echinaster crassispina* showed a like tendency to right away from the ray handled, but here the results were not so invariable, in fact, one specimen was found in which the ray handled was used to right upon, and this same behavior continued even when the handled ray was changed.

SUMMARY

1. Generally, in locomotion, one ray is not used as a director more than another.

2. The tube-feet may act as temporary suckers and thus may enable the ophiuroid to climb vertical walls.

3. The tube-feet react to food and may carry this food to the proximal end of the ray even when the latter is severed from the rest of the animal. Inorganic objects are not handled in this way.

4. Experiments show that the twisting of the rays at the proximal end is an important factor in righting.

5. The ophiuroids studied show that the contact of a ray with a solid wall is often an important factor in determining the direction of locomotion and that the effect of the stimulus produced is retained for some time.

6. This so-called "memory of past stimulation" seems to be lost when the ophiuroid comes under the influence of a stronger stimulus, such as bright sunlight, coming from one general direction.

7. Under the latter conditions the direction of locomotion is almost always negative and quite definite. It is, however, not stereotyped.

8. The ophiuroids studied right themselves away from bright light and then continue to move away from it; on the other hand, the starfish studied rights itself away from the bright light and then moves toward it.

9. The contact of a ray or rays with a solid wall before the specimen is inverted often has some effect on the direction of righting.

10. A shadow cast on the floor on which an ophiuroid is moving does not seem to act as so strong a stimulus as the shadow produced in a cavity.

11. Ophiuroids undoubtedly react to dark vertical walls even when they cast no shadow.

12. The method of handling an ophiuroid often determines the direction of locomotion and righting.

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THE EFFECTS OF CENTRIFUGAL FORCE UPON THE ORGANIZATION AND DEVELOPMENT OF THE EGGS OF FRESH WATER PULMONATES

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FORTY-SEVEN FIGURES

In one of his earliest papers Professor Brooks (1879) described the early stages in the development of *Physa*, *Lymnaea* and *Planorbis*, devoting particular attention to the cleavage of the egg, the formation of the germ layers and the history of the digestive tract. This work was done on living material, observations being made upon eggs within the capsules, and he says (p. 77):

I have used every effort to actually see the living egg pass through all the stages of development. To do this requires such close and constant attention that it leaves no time for the more minute study of eggs which have been treated with reagents, and I have no observations to offer upon the fascinating subject which is now receiving so much attention from embryologists—the history of the changes which result from fertilization, and the origin and behavior of the segmentation nuclei. On the other hand the observation of the changes of the living egg from one stage to another, brings into prominence certain features which would be entirely lost sight of in the study of a series of preserved specimens.

Professor Brooks observed that a few minutes after the egg is laid the germinative vesicle becomes invisible and that a clear watery fluid appears at the pole of the egg at which the polar body is forming. He believed that this clear fluid disappeared subsequently during the cleavage. Regarding the different substances which may be seen in the egg he says (p. 80), "The end of the second period of rest (4-cell stage) is of especial interest, since we find that the segregation of the protoplasm of

the endoderm and ectoderm now takes place, and is plainly shown before the commencement of the next period of activity which is to result in the separation of the macromeres from the micromeres. In a side view of the egg at the end of the second period of rest, fig. 11, each spherule is divided into three pretty well marked regions, distinguished from each other by the amount of deuteroplasm contained in the protoplasm. The formative end of each spherule is quite transparent and contains none of the large spherules of deuteroplasm, and only a few granules. The central portion of each spherule is quite granular and contains many of the food spherules, but it is much more transparent than the yolk of the unsegmented egg. A large nucleus is also very conspicuous in this region. Almost all of the food material is massed in an opaque ball at the nutritive pole of each spherule.

In subsequent stages he observed the formation of two sets of micromeres, containing the transparent substance at the formative pole, also the macromeres composed largely of food substance, and he determined that the ectoderm came from the former and endoderm from the latter.

The year in which this paper by Professor Brooks appeared also witnessed the publication of Rabl's (1879) beautiful and extensive work on the development of Planorbis. He observed that the protoplasm of the freshly laid egg showed a significant polar differentiation, the animal half being composed of small and the vegetative half of large yolk granules; in reflected light the former appears whitish, the latter yellow. He followed the cleavage in detail and showed that at the 24-cell stage there exists a complete separation of the three kinds of elements out of which the germ layers take their origin; and after showing that this distinction in these elements is recognizable in the 8-cell, the 4-cell and the 2-cell stages, he says (p. 572):

Unwillkürlich drängt sich uns nun die Frage auf, ob wir nicht auch schon in der ungefurchten Eizelle eine ganz bestimmte und gesetzmässige Anordnung und Vertheilung der Protoplasma-Partikelchen und Moleculen anzunehmen haben. Und in der That, eine solche Annahme erscheint uns viel wahrscheinlicher, als etwa die in jüngster Zeit von Goette ausgesprochene Ansicht, dass das Ei eine todte, unorganisirte Masse sei, oder aber als die von manchen Forschern, die sich gern mit

ihrem Monismus oder Materialismus brüsten, mit besonderer Vorliebe vertretene Ansicht, dass das Ei nichts weiter, als ein Klümpchen höchst einfacher protoplasmatischer Substanz sei, aus der sich erst später die einzelnen Molecüle wie aus einem chaotischen Wirrsal nach ihren gegenseitigen "Verwandschaften" zusammenfänden. Als ob mit solchen und ähnlichen Behauptungen die Schwierigkeiten, welche der Erklärung einer entwicklungsgeschichtlichen Erscheinung im Wege stehen, beseitigt werden könnten! Als ob nicht schon in dem Worte Protoplasma allein das Räthsel des Lebens und der Entwicklung läge! Müssen wir aber schon das Protoplasma jeder Muskel- oder Nervenzelle als eine ausordentlich complicirte Substanz betrachten, um wie viel mehr erst das Protoplasma der Eizelle, aus der die verschiedensten Gewebe des Körpers ihren Ursprung nehmen.

In another pulmonate gasteropod, *Limax campestris*, Mark (1881) found in the eggs, just after they were laid, an immense number of granules held in suspension in a viscid transparent protoplasm. These granules are irregularly distributed, producing a cloudy effect, and there is a thin shell of protoplasm at the surface entirely destitute of granulations. Within the egg an elongated lighter portion, free from granules, marks the position of the first maturation spindle. The movement of this clear area to the surface and the spread of this clear protoplasm at the animal side of the egg is excellently described. During the first cleavage of the egg he observed in some cases an equatorial zone of protoplasm from which the granules are eliminated. The outlines of this zone, presented in optical section, is that of two narrow wedges, with their bases at the surface and their apices directed toward and nearly reaching each other (p. 183). I have observed a similar phenomenon in *Physa* and *Planorbis*, to which reference will be made later.

Later writers, notably Kofoid (1895), Meisenheimer (1896), Holmes (1900), and Wierzejski (1905), have added greatly to our knowledge of the maturation, fertilization and cleavage of the eggs of pulmonate gasteropods, but they have dealt only secondarily, if at all, with the question of the different egg substances and their distribution.

OBSERVATIONS

My own observations on the nature and movements of the egg substances of pulmonates are not very different from those of Brooks, Rabl and Mark. In 1902 I spent several weeks in the study of the phenomena of maturation, fertilization and cleavage, as they may be seen in the living eggs of these animals. In the main these observations were confined to the eggs of *Physa heterostropha*, *Lymnaea columella*, and *Planorbis trivolvis*. In all of these the phenomena observed are essentially alike and, unless otherwise specified, it may be assumed that the following account applies to all the species named.

At the time the eggs are laid there is a large spherical germinal vesicle in the egg, which is quite transparent, whereas the rest of the egg is rather opaque from the presence of yolk granules and a diffuse yellow pigment, which is uniformly distributed throughout the egg (fig. 1). At this stage there is no evident distinction between the substances at the animal and vegetative poles. The germinal vesicle is slightly eccentric toward one pole, but neither at this stage nor at any subsequent one do the eggs orient themselves with respect to gravity, consequently they lie in the jelly masses with their chief axes in almost every possible direction.

As the germinal vesicle begins to dissolve and the first maturation spindle appears the clear area of the germinal vesicle becomes elliptical and then spindle-shaped in outline. This clear area then moves through the egg until one end of the elongated area comes into contact with the surface at the animal pole of the egg, leaving a deep "well" of clear protoplasm leading down to the center of the egg (fig. 2). Close around this clear protoplasm is a finely granular yellow substance which is quite distinct from the yolk on the one hand and the clear protoplasm on the other. As the first maturation division advances this "well" grows shallower and the clear protoplasm is spread out on the surface as a cap at the animal pole (figs. 3, 4). This cap is pearl gray or milky in appearance when seen by reflected light, or when brightly illuminated by transmitted daylight; it will be referred to as the clear protoplasm or substance.

The first polar body is extruded about two hours after the egg is laid. At the moment of its extrusion the outline of the entire egg becomes irregular and a "yolk lobe" frequently, if not invariably, appears at the vegetal pole. The second polar body is formed about one hour after the first; during its formation the clear protoplasm is spread out as a cap at the animal pole, and no deep "well" of such protoplasm is found as during the first maturation (figs. 4, 5). The egg again becomes irregular in outline at the moment the polar body is extruded and a yolk lobe is formed. The appearance of the living egg before and during these maturation divisions suggests the view that during the nuclear division the surface tension of the egg is increased, giving rise to what Reinke (1900) has called "mitotic pressure;" and that this tension is reduced the moment the polar body begins to be extruded.

During and immediately after the second maturation division the cap of clear protoplasm at the animal pole begins to spread over the upper hemisphere (figs. 5-8), and at the time of the first cleavage it has reached the equator of the egg (fig. 9, 10). The advancing edge of this cap becomes thickened, so that in optical sections it frequently has the appearance of extending nearly through the egg (fig. 11), as described by Mark.

When this clear cap has extended about half way to the equator, the sperm nucleus may be seen approaching the egg nucleus from the lower pole, both appearing as clear spots in the egg. They meet at the animal pole, where they lie in a deeper portion of the clear protoplasm. Immediately beneath these nuclei is a layer of finely granular yellow substance, which later surrounds the nuclei and the cleavage spindle (figs. 7, 8). From its position relative to the nuclei and spindle there can be little doubt that this substance corresponds to the "archoplasm" of *Ascaris* (Boveri 1887), or the "sphere material" of *Crepidula* (Conklin 1902). The germ nuclei then appear to elongate, the wall between the two nuclei disappears and the two asters of the first cleavage spindle become visible as clear areas at the ends of the first cleavage spindle. Even after the central portion of the spindle has ceased to be visible the asters may still be seen as clear areas in the egg.

The first cleavage furrow then begins to appear. A "cleavage head" (Ziegler 1898) of clear protoplasm cuts into the egg over the upper hemisphere in the plane of the first cleavage (fig. 10). In this plane the clear protoplasm extends around to the vegetative pole and the constriction then deepens all around the egg, and at the same time the line of demarcation between the clear and yellow hemispheres becomes indistinct. Finally the constriction is completed and each daughter cell becomes an almost perfect sphere, in contact with its sister by only a small area. The daughter nuclei appear as clear spots, the clear astral areas disappear and in their places the yellow sphere substance is aggregated, and the line of demarcation between the clear and yellow hemispheres of the egg becomes more distinct (fig. 11). Finally the portion of the yellow pole uncovered by the clear protoplasm becomes smaller, the daughter cells flatten together until they are nearly hemispherical in shape and the first cleavage is completed.

At the close of the first cleavage a small lenticular space makes its appearance in the cleavage plane between the daughter cells, apparently from the region of the mid body ("Zwischenkorper"), and it increases in size until it extends nearly to the periphery of the egg (figs. 12,13). This is the "intermittent cleavage cavity" of earlier investigators.

The second cleavage then begins; the mitotic figure appears as a clear elliptical area surrounded by the yellow sphere substance (fig. 13); the lenticular cleavage cavity disappears and the clear protoplasm, which covers the entire upper hemisphere, may be seen to dip down into the first cleavage plane in the place of this cavity (figs. 13, 14). The second cleavage is completed in the same manner as the first.

From the 4-cell stage onward the substance of the yellow hemisphere is gradually encroached upon by the clear protoplasm until it is limited to a small area around the vegetal pole (fig. 15). The first, second and third sets of micromeres (ectomeres) are formed and they contain little, if any, of this yellow substance, all of which is left in the macromeres (figs. 16-21). In all subsequent divisions of the macromeres to form the mesoderm and endoderm cells of the fourth quartet, this yellow substance is distributed

to all of these cells (fig. 22). Finally as the ectoderm cells of clear protoplasm overgrow the endoderm and mesoderm cells, the yellow material is entirely removed from the surface.

In the course of development, from the maturation of the egg to the gastrulation, the relative quantities of clear and yellow substance are reversed. At the beginning the clear substance is small in quantity, and is chiefly visible in the germinal vesicle, (though experiments show that some of it is distributed through the yellow substance) and at this stage the entire cell body is yellow in color. With the establishment of the germinal layers the yellow substance is limited to the few cells constituting the endoderm and mesoderm, while all the rest of the embryo, by far the larger part, is composed of clear substance. This change in the relative quantities of these two substances is due in part to their separation and segregation during the course of development but in much greater part to the transformation of yellow substance into clear.

It is a phenomenon of general occurrence among many animals that the clear protoplasm of the egg is very small in quantity before the dissolution of the germinal vesicle and that it gradually increases in quantity after that stage. This is doubtless due in large part to the dissolving of yolk and its conversion into clear protoplasm, and it is a significant fact that this process takes place most rapidly after the breaking down of the wall of the germinal vesicle and the escape of a large part of the nuclear contents into the cell body. Whether the clear substance which normally goes into the ectoderm cells, and the yellow material which constitutes the endoderm and mesoderm cells are totipotent, or specifically differentiated histogenetic materials can be determined only by experiment.

EXPERIMENTS

Preliminary observations on the effects of pressure on these eggs were made in 1902, but no extended experiment were undertaken until 1909. From April 3 to June 2 of that year a large number of experiments were made on the eggs of *Physa ancillaria*

Say, and of *Lymnaea catascopium* Say in order to determine more fully, than was possible by observation alone, the nature of the egg organization and the potency and significance of the different substances in these eggs. The principal method of experiment was that of centrifuging the eggs at different stages of development and to varying degrees. The freshly laid eggs were placed while still in their jelly in small tubes which were whirled on a centrifugal machine driven by water pressure. The radius of rotation was 6 cm., and the average velocity 3000 revolutions per minute, from which data the centrifugal pressure may be calculated to be approximately 600 times that of gravity.

Since each egg is contained within a capsule which does not collapse under the pressure used, and since these capsules are contained within jelly masses which keep the capsules well separated from one another, all danger of distortion due to mutual pressure is avoided. The eggs are free at all times to rotate within the capsules; at the same time the capsules are easily isolated, they afford a perfectly normal environment for the further development of the eggs, and if substances are separated from the egg in the process of centrifuging the further history of these substances, as well as the eggs from which they came may be followed through the entire course of development until the adult form is reached and little snails escape from the capsules. All of these features make these eggs unusually favorable for such experiments.

Eight separate egg-masses, containing from 25 to 75 eggs each, were centrifuged in the germinal vesicle stage. In a few instances the eggs were taken just as they were being laid, in other cases just before the disappearance of the germinal vesicle, and certain slight differences in the results of centrifuging different lots of eggs may probably be attributed to this slight difference in the stage at which the centrifuging occurred. In all of these cases the relative age of the eggs may be determined by observing the time at which the first polar body appears, since this usually occurs about two hours after the egg is laid. In the following account the experiments are described in the order of the age of the eggs at the time they were centrifuged, the earliest stages

coming first and the latest last. It will be understood without further mention that in this account *Physa* refers to *Physa ancillaria* Say; and *Lymnaea* to *Lymnaea catascopium* Say.

Experiment 1. A large egg-mass of *Lymnaea* was taken just after it was laid, and centrifuged for 10 min. in the germinal vesicle stage. After centrifuging the eggs were found to be slightly elongated or elliptical (fig. 23), the narrower end being central, and the broader end distal (peripheral) in the tubes. The egg substances were separated into three very distinct zones, gray, transparent (clear), and yellow; the gray substance was finely granular in structure and occupied the narrower (central) end of the egg; the clear substance was almost perfectly transparent and constituted the middle zone; while the yellow material contained yolk spherules as well as yellow pigment and occupied the broad end of the egg. The gray and clear materials together constituted about one-half of the egg substance, the gray forming about three-eighths, and the clear one-eighth, while the yellow substance made up the other one-half. In all cases the germinal vesicle lay in the clear zone. The yellow zone was so much heavier than the other two that it turned to the lower side before it could be examined under the microscope; it was therefore necessary to tilt the microscope into a horizontal position, with the stage and slide in a vertical position in order to see these three zones. Half an hour after the eggs were removed from the centrifuge they all became pear-shaped, or unequal dumb-bell shaped; the gray substance occupying the small end, the yellow the large end and the clear substance lying in the neck between the two (fig. 25). In many cases this neck became so constricted that the two ends became nearly or quite separated. One hour and fifty minutes after the eggs were centrifuged the first polar body appeared, usually on the clear zone (fig. 26), but sometimes on the edge of the yellow or gray zone adjoining the clear one. At the same time the clear zone was seen to be less sharply separated from the gray and the yellow than was the case immediately after centrifuging. The eggs still oriented as before with the yellow pole down and the gray pole up. Five hours after centrifuging the eggs had passed into the 2-cell

stage. The gray and yellow poles could still be distinguished, but the clear zone between them had almost disappeared, and the eggs no longer oriented rapidly, if at all. The yellow and gray poles of the egg occupied any conceivable relation to the first cleavage plane, with the result that one of the first two cells might be gray, the other yellow (fig. 35), or the gray and yellow substances might be distributed equally to the two cells (fig. 39), or there might be many intermediate conditions between these two extremes (fig. 43). If the distribution of these substances was unsymmetrical in the first two blastomeres, it remained unsymmetrical throughout the whole course of development. The cleavage forms were usually regular and normal except for the distribution of the yellow and gray materials, and a blastula, gastrula and larva of normal form developed from every egg, though the yellow and gray substances might occupy any position in the embryo or larva. Finally after fifteen days all of the young except one, some 40 in all, had hatched, and all were perfectly normal little snails.

Three eggs of this lot were isolated in the 2-cell stage, being selected because they showed very unsymmetrical distribution of the yellow and gray substance. They continued to develop for from 3 to 15 days but all of them died before hatching. In view of the fact that all the other eggs in this experiment developed into normal snails it seems probable that these isolated eggs failed to develop because of some injury due to isolating them, or to some injurious substance in the dishes in which they were placed.

Experiment 2. A *Lymnaea* egg-mass was centrifuged 10 min. about 20 min. after the eggs were laid, and while all were still in the germinal vesicle stage. The separation of the egg substances into gray, clear and yellow zones occurred as in the preceding experiment. The first polar body was extruded 1 hr. and 30 min. after the centrifuging, and in every case (37 eggs) from the clear zone. The eggs oriented as in the preceding experiment, and in order to see the three zones under the microscope it was necessary to tilt the stage into a vertical position. The second polar body was extruded 1 hr. and 15 min. after the first, usually from the clear zone, though the latter had mingled considerably

with the gray and yellow zones by this time, so that the distinction between them was not so sharp as at first. The eggs still oriented with the gray pole up, the yellow pole down and with the polar bodies on the surface between the two. However, before the first cleavage, which occurred about 2 hrs. after the formation of the second polar body, the clear zone was but faintly distinguishable from the gray and yellow and the eggs no longer oriented rapidly as they did earlier. The axis connecting the gray and yellow poles (axis of stratification) may lie at any angle with respect to the first cleavage plane, and consequently the gray and yellow substances may be distributed equally to the first two blastomeres or all the gray may go into one of these blastomeres, and all the yellow into the other, or all degrees of unequal distribution of these substances may occur. This unsymmetrical distribution of gray and yellow material could be recognized at all stages of the cleavage and gastrulation; however, the form of the cleavage, the gastrula, and the veliger were entirely normal, and every egg of this lot, without exception, gave rise to a perfectly normal snail.

Nine eggs of this lot were isolated in the 2 and 4-cell stages; all of these showed abnormal distribution of the gray and yellow substances and yet every one of them gave rise to a normal snail.

Experiment 3. Eggs of *Lymnaea*, in the germinal vesicle stage, but some time after being laid, were centrifuged for 10 min. The stratification of substances, orientation of eggs and place at which the polar bodies were extruded was the same as in the preceding experiments. In the gastrula stage, however, some were seen to be abnormal in form as well as in the distribution of the gray and yellow substances, and after 14 days 11 were abnormal or dead, while 46 were apparently normal.

Experiment 4. Eggs of *Lymnaea* in the germinal vesicle stage and about 1 hr. before the formation of the first polar body, were centrifuged, full speed, for 20 min. The separation of the substances was very sharp, the relative quantities being about as follows: gray one-eighth, clear three-eighths, yellow one-half. The germinal vesicle always remained in the clear zone, though it sometimes encroached a little upon the yel-

low or the gray zones and it usually, if not invariably, lay out of the axis of stratification. About 40 min. after centrifuging the germinal vesicle gave rise to a clear spindle-shaped figure, the first maturation spindle, which usually lay oblique to the axis of stratification, but in some instances was at right angles to it. One end of this figure came into contact with the surface of the egg, usually near the boundary between the clear and gray zones (as in figs. 24,25), and here the first polar body was extruded about 1 hr. after the eggs were removed from the centrifuge. Just before the extrusion of the polar body the eggs became pear-shaped, the gray substance occurring in the narrow end of the pear, the clear substance in the constricted region, or neck, and the yellow in the larger end. The second polar body was usually extruded near to, or immediately under the first. The centrifuged eggs oriented as in the preceding experiments, with the gray pole up and the yellow pole down. When floating freely in water on a horizontal stage 20 eggs showed the polar bodies on the side while 5 did not. When viewed from the side, with the stage in a vertical position, the polar bodies were seen to be on the clear zone in 10 eggs, on the edge of the gray zone, near the clear, in 3 eggs, and on the edge of the yellow zone in 2 eggs, while the polar bodies were invisible, probably lying over or under the egg so that they could not be seen, in 10 eggs. Therefore, in 25 eggs all but 5 had the polar bodies on the clear zone, and these lay near the clear zone on the gray or yellow. The cleavages of these eggs were normal in form though generally abnormal in the distribution of the gray and yellow substances. The gastrulae were also normal save for the unsymmetrical distribution of these substances, and at the end of 12 days all of the young snails except one had hatched, and all were normal, or nearly so.

Two eggs of this lot were isolated in the 2-cell stage, one of the blastomeres containing all of the gray substance and the other all of the yellow. The cleavage, blastula and gastrula stages were normal in form, though abnormal in the distribution of these substances, and normal snails hatched from the capsules in both cases.

Experiment 5. *Lymnaea* eggs were centrifuged for 10 min., generally after the disappearance of the germinal vesicle, but before the formation of the first polar body. The three zones were quite distinct, the gray pole floated uppermost and the eggs oriented rapidly. In one or two eggs the germinal vesicle was still intact after centrifuging, lying in the clear zone, but bordering on the gray. The first polar body formed 1 hr. and 25 min. after centrifuging; at this time the gray and yellow zones were still fairly distinct but the clear zone had largely diffused into the other two. In most cases the gray pole still floated up, and the yellow pole down. The first polar body was always on some portion of the gray zone, but usually near the remains of the clear zone, consequently many eggs, after they had oriented, showed the polar body on the side. On the 4th day the embryos were normal except for the distribution of the gray and yellow materials; on the 9th day normal little snails were present, but not yet hatched from the capsules; on the 16th day all had hatched as normal snails.

Experiment 6. *Lymnaea* eggs, from a part of the same laying used in Exp. 1, were centrifuged 10 min. just as the first polar body was being extruded. The separation of the yellow, clear and gray substances was fairly complete. The first polar body always formed on the gray cap, though often eccentrically. The eggs did not orient as rapidly as in Exp. 1, but they still oriented in the same way. The gray substance was more coarsely granular than in Exp. 1, and in general it was equally abundant with the clear and yellow substances, each constituting about one-third of the volume of the egg. On the 2d day few were normal, many abnormal or dead. The same was true on the 8th and 12th days. Almost all left alive on the 15th day were normal (The exact proportion of normal to abnormal forms was not noted.)

Four eggs of this lot were isolated in the 2-cell or 4-cell stages, as follows:

1. Two cells, one yellow the other gray (as in fig. 35). Subsequent cleavage stages were normal in form but not in distribution of colored materials; the same was true of the blastula, one-half of which was yellow and the other half gray. On the

3d day a gastrula was present, normal in form, but with the anterior half yellow and the posterior half gray. On the 8th day this egg had given rise to a normal little snail, still unhatched; and on the 12th day it hatched and appeared entirely normal.

2. Isolated in the 4-cell stage, the four cells being of a uniformly yellow tint, while the gray material was contained in a large spehre about one-fifth the volume of the entire egg. This sphere had been extruded at the animal pole and was really the second polar body, and upon it the first polar body was attached (figs. 27-29). On the 2d day the blastula stage was reached it was of a uniformly yellow color but was otherwise quite normal. On the 3d day the gastrula was formed (fig. 30) which was chiefly yellow in color, though the ectoderm was more transparent than the endoderm. Daily examinations showed that the development of this embryo was entirely normal and on the 12th day a normal snail hatched from the capsule. The gray sphere (second polar body) took no part in this development, but died soon after its separation from the remainder of the egg.

3. An egg exactly similar to the preceding, gave rise on the 2d day to a large number of blastomeres which were loosely united, and which did not develop further, though observed up to the 8th day. The gray sphere never divided and took no part in the development.

4. An egg similar to (2) was isolated in the 4-cell stage. The gray sphere (second polar body) in this case was very large, comprising almost one-half the volume of the entire egg. On the 2d day the gray sphere appeared to be composed of many small spherules, but with outlines so obscure that it was difficult to see them at all; after the 2d day no further development of the gray sphere occurred. On the 3d day the yellow part of the egg had given rise to a gastrula, yellow in color but with ectoderm more transparent than the endoderm. Subsequent daily examinations showed that the development of this embryo was entirely normal, except for size and color, until on the 12th day a normal little snail hatched and lived for many days.

Experiment 7. *Lymnaea* eggs after both maturation divisions and 1 hr. before the first cleavage were centrifuged 20 min. full

speed. Either the yellow or the gray pole floated up, though more had the yellow pole down than up, *i.e.*, they oriented as in earlier experiments, but more slowly. The polar bodies were at the yellow pole, or the gray pole or anywhere between the two, *i.e.*, the axis of stratification formed no constant angle with the chief axis of the egg, and consequently the eggs did not orient while on the centrifuge. In general the distribution of the yellow and gray materials to the first two blastomeres was very abnormal and in some cases the form of the cleavage was abnormal also. On the 12th day 13 snails were normal, 8 were abnormal, and 14 were dead.

Experiment 8. Lymnaea eggs were centrifuged 10 min. just before the first cleavage. The separation of substances was not as complete as in earlier stages, and the eggs oriented very slowly if at all. After 10 days many were dead, a few were unhatched but living. After 12 days 9 of the latter were dead, and 2 living, but abnormal and unhatched.

Experiment 9. Lymnaea eggs, at the time of the first cleavage (some eggs divided, others undivided) were centrifuged 10 min. The egg substances separated into yellow and white zones the latter composed of the clear and gray substances. Orientation, with the white pole up and the yellow down, took place very slowly, if at all. In many cases the yellow substance was particularly dense around the nuclei and spindles. The white substance covered about seven-eighths of the egg, and within it were many yolk granules; there was no finely granular gray substance as was the case when eggs were centrifuged in the maturation stages. On the 3d day a gastrula was formed, nearly normal in form; on the 4th day ciliated embryos were present; on the 9th day many young snails were more or less abnormal; on the 13th day none had hatched and many were abnormal; on the 16th day 4 were normal and had hatched, 40 were more or less abnormal and unhatched.

Experiments 10 to 22 were made on the eggs of *Physa ancillaria* Say.

Experiment 10. Eggs centrifuged 12 min. in the germinal vesicle stage, the latter being present in the clear substance after

centrifuging. The first cleavage appeared 4 hrs. later, the distribution of substances to the blastomeres being abnormal in many cases. On the 5th day young ciliated veligers were present and apparently normal. On the 7th day the entire lot accidentally dried up.

Experiment 11. Eggs of *Physa* in the germinal vesicle and first polar body stages (most of the eggs had just extruded the first polar body, while others were still in the germinal vesicle stage), were centrifuged for 5 min. The stratification into gray, clear and yellow substances was rather incomplete, with a general preponderance of yellow. Many of the eggs showed a lobe of gray substance, though none of them were markedly elliptical, and all were irregular in form. At the first cleavage the eggs divided into two equal blastomeres, and the distribution of substances was generally symmetrical, though unsymmetrical in a few cases. On the 2d day the blastulae, on the whole, appeared normal; while on the 9th day all had developed into normal snails and some had hatched.

Experiment 12. *Physa* eggs, which were pear-shaped and were probably in the act of extruding the first polar body, were centrifuged $3\frac{1}{2}$ min. The subsequent development for the first 6 days was apparently normal, except for the distribution of the colored substances. The latter stages of development were normal.

Experiment 13. *Physa* eggs were centrifuged for 5 min. after the formation of the first (and in a few cases possibly of the second) polar body. When removed from the centrifuge the eggs were elliptical in shape and the three zones were sharply defined. The yellow material was much less abundant than the gray, and the eggs oriented rapidly, with the yellow pole down. In the 2-cell stage the distribution of the colored substances was, in most cases, symmetrical. On the 8th day little snails had developed which seemed normal, but had not yet hatched.

Isolations. 1. A centrifuged egg was isolated in the 2-cell stage; one cell was gray, the other yellow. In the 4-cell stage two of the cells were gray and two yellow. On the 2d day a blastula, nearly normal in form, had developed, but one-half was yellow and the other half gray. Subsequent daily examination showed that the

development was normal except for the distribution of the colored substances, and on the 11th day a large normal snail hatched from the capsule.

2. Another centrifuged egg was isolated in the 2-cell stage, the stratification of the materials being oblique to the cleavage plane, so that one cell was chiefly gray and the other chiefly yellow. (as in fig. 43). The subsequent cleavage was normal in form and on the 2d day an irregular blastula developed, one pole of which was clear, the other yellow, while a crescentic area of gray substance lay between the two. On the 8th day a normal little snail had developed and on the 11th day it hatched from the capsule as a large, normal snail.

3. A centrifuged egg, in which one cell was gray and the other chiefly yellow, was isolated in the 2-cell stage. This segregation of the colored materials persisted through the early cleavages and on the 2d day an irregular parti-colored blastula developed. On the 8th day the embryo was dead.

4. Another egg in which the stratification was oblique to the first cleavage plane was isolated in the 2-cell stage, one of the cells being gray and the other gray and yellow. In the 4-cell stage three cells were predominantly gray and one yellow. On the 8th day a normal snail hatched from the capsule.

Experiment 14. Centrifuged 5 min. after the extrusion of both polar bodies and 30 min. before the first cleavage. In the 2-cell stage one blastomere was often yellow and the other gray, in the 4-cell stage two were frequently yellow and two gray. An embryo abnormal in form and in distribution of the egg substances usually resulted. On the 8th day 42 were apparently normal but unhatched, 6 were abnormal and unhatched, and 32 had died about the gastrula stage. On the 12th day many of the apparently normal forms had hatched and were quite normal.

Experiment 15. Eggs of *Physa* were centrifuged 22 min. after the formation of both polar bodies, in the vain attempt to separate the gray material as an exovate. The eggs were drawn out into long cylinders (fig. 31), the proportions of the three substances being about as follows: Gray one-half, clear one-quarter, yellow one-quarter. The distribution of these substances in the

cleavage was abnormal, and the form of the cleavage, blastula and gastrula was generally abnormal (figs. 32, 33). On the 11th day 8 young snails were nearly normal but unhatched, 3 were very abnormal and unhatched, 15 had died about the stage of the gastrula. Two of the 8 nearly normal snails had conical shells with the apex turned forward and to the right (fig. 47); whereas in normal *Physas* it turns back (posteriorly) and to the left.

Experiment 16. Centrifuged 12 min. after the formation of both polar bodies and before the first cleavage. After centrifuging the eggs became elongated and the subsequent cleavage was frequently very abnormal in form as well as in the distribution of substances. The blastulae and gastrulae were generally abnormal in form; on the 8th day all of 25-30 embryos were dead except 4.

Isolations. Thirteen of these centrifuged eggs were isolated in the 2-cell stage for various abnormal distributions of substances, and all of them died about the gastrula stage.

Experiment 17. Centrifuged 12 min. after the extrusion of both polar bodies. The stratification of substances was distinct, but the eggs did not elongate as in Exp. 16. In the 2-cell and 4-cell stages the distribution of the yellow and gray substances was very abnormal (as in figs. 32-33). On the 10th day 3 young snails had hatched and were quite normal, 1 was unhatched but nearly normal, 2 were unhatched and very abnormal and 27 had died about the gastrula stage.

Experiment 18. Centrifuged for 2 min. after the formation of the polar bodies and before the first cleavage (exact age unknown). The separation of the egg substances was not very distinct. On the 10th day 22 young snails had hatched, 17 had not hatched, and 43 had died about the gastrula stage.

Experiment 19. Centrifuged 5 min. just before the first cleavage of the egg. The gray substance was in all cases more abundant than the yellow, and in all cases in which the distribution of these substances to the first two blastomeres was unsymmetrical the yellow cell was smaller than the gray (as in fig. 32). At the 4-cell stage of many eggs, two cells were small and yellow, while two were large and gray (as in fig. 33). At the 8-cell stage each

of these cells gave rise to a micromere, two of which were yellow and two gray. On the 3d day blastulae had developed, which were abnormal in form and distribution of substances. On the 9th day 17 were normal and had hatched, 2 were abnormal and were unhatched, 27 were dead in the gastrula stage. The 2 abnormal snails showed exostomata, the buccal cavity and radula being evaginated, so that the lingual ribbon was spread out on the surface (fig. 46).

Isolations. Five eggs of this lot were isolated in the 2-cell stage. Four of these showed the same type of abnormal distribution of substances, viz., all the yellow material was contained in a small cell, all the gray in a large one. The yellow material was dense and finely granular, the gray contained large granules and yolk spheres. Blastulae and gastrulae, abnormal in form and distribution of colored substances, were formed. Four of these isolated embryos died about the gastrula stage; one lived and on the 9th day gave rise to a large normal snail, which hatched from the capsule. In the early stages there was no evident difference between the eggs which did not develop beyond the gastrula and the one which did.

Experiment 20. Eggs in the germinal vesicle stage were centrifuged slowly for 4 hrs. up to the time of the first cleavage, at the close of which time the separation of substances was very complete and the eggs much elongated. On the 4th day abnormal and irregular gastrulae had formed; on the 5th day ciliated embryos were present and rotating; on the 7th day veligers had formed and looked approximately normal; on the 16th day none had hatched and all were very abnormal.

Experiment 21. Centrifuged 10 min. in the 2-cell stage. The early cleavages were normal in form, although not in the distribution of substances. All had died on the 2d day.

Experiment 22. Centrifuged 10 min. in the 2-cell stage. The cleavages were very abnormal in form and in distribution of substances; the blastulae and gastrulae were parti-colored and irregular in form; and all had died on the 5th day.

These experiments are briefly summarized in the tables on the pages following.

The experiments on the eggs of *Physa* were less detailed and exact than those on the eggs of *Lymnaea*. Several of the *Physa* experiments were made early in my work before I had fully appreciated the importance of detailed records as to the exact stage of the eggs at the time of centrifuging, the relative quantities and weights of the different substances, the positions of the polar bodies with reference to the different zones, and the precise number of normal as compared with abnormal embryos. Furthermore the eggs of *Physa* were more easily injured than those of *Lymnaea*, so that the length of time during which the eggs were centrifuged was varied considerably in the hope of finding the most favorable period, and these facts must be taken into account in comparing the results of these different experiments. On the whole, however, a comparison of the two tables shows that the effects of centrifuging were essentially similar in the two genera.

CONCLUSIONS

The principal results of these experiments is to show that important changes take place in the oöplasmic substances during the interval between the first maturation and the first cleavage. These changes concern the relative quantities and weights of the three principal substances, and the dissimilar effects of centrifuging at different periods. These experiments also throw light upon questions of the polarity and symmetry of the egg and the potency of the oöplasmic substances.

1. The relative proportions of gray, clear and yellow substances undergo great changes during the period between the first maturation and the first cleavage. Before the first maturation the yellow substance composes at least one-half of the entire egg; just before the first cleavage it composes only about one-eighth of the egg. The clear and gray substances, which together constitute about one-half of the egg in the earlier period, form seven-eighths of the egg in the later period. In early stages the yellow substance contains the yolk spherules and, in strongly centrifuged eggs, consists almost entirely of these; after the dissolution of the germinal vesicle much of the yolk disappears and

TABLE 4
Results of centrifuging eggs of *Limnaea catascopium*, Say

XP. NO.	CENTRIFUGED Stage	STRATIFICATION OF SUBSTANCES				POLAR BODIES ON			ORIENTA- TION	CLEAVAGES		GASTRULAE		YOUNG SNAILS		
		Duration	Gray	Clear	Yell ^w	Axis	Gray	Clear		Yellow	Form	Subs.	Form	Subs.	Normal	Abnor.
1	Just laid Germ. vesicle	10 min.	$\frac{1}{2}$	$\frac{3}{4}$	$\frac{1}{2}$	Any	0	All	0	Rapid	Norm.	Abnor.	Norm.	Abnor.	40	1
2	Laid 20 min. Germ vesicle	10 min.	$\frac{1}{2}$	$\frac{3}{4}$	$\frac{1}{2}$	Any	0	37	0	Rapid	Norm.	Abnor.	Norm.	Abnor.	37	0
3	Germ. vesicle	10 min.				Any	Almost all			Rapid			Some	Abnor.	46	11
4	Germ. vesicle, 1 hr. before 1st polar body	20 min.	$\frac{1}{2}$	$\frac{3}{4}$	$\frac{1}{2}$	Any	3	20	2	Rapid	Norm.	Abnor.	Norm.	Abnor.	Many	1
5	After germ. ves., before 1st pole b.	10 min.					All	0	0	Fairly rapid	Norm.	Abnor.	Norm.	Abnor.	All	0
6	1st. pole body forming	10 min.	$\frac{1}{2}$	$\frac{3}{4}$	$\frac{1}{2}$		All	0	0	Less rapid	Many	Abnor.	Many	Abnor.	Many	Many
7	After both pole bodies, 1 hr. before 1st cleavage	20 min.				Any	On	Any	Zone	Slow	Many	Abnor.	Many	Abnor.	13	22
8	Just before 1st cleavage	10 min.	$\frac{1}{2}$	$\frac{3}{4}$	$\frac{1}{2}$	Any	On	Any	Zone	Very slow	Many	Abnor.		Abnor.	0	Many
9	At time of 1st cleavage	10 min.	$\frac{1}{2}$	$\frac{3}{4}$	$\frac{1}{2}$	Any	On	Any	Zone	Very slow	Many	Abnormal.			4	40

TABLE 2
Results of centrifuging eggs of *Physa ancillaria*, Say

EXP. NO	CENTRIFUGED		STRATIFICATION OF SUBSTANCES				CLEAVAGES		GASTRULAE		YOUNG SNAILS	
	Stage	Duration	Gray	Clear	Yell ^w	Degree	Form	Subs.	Form	Subs.	Normal	Abnormal
10	Germ. vesicle, 4 hrs. before 1st cleavage	12 min.				Distinct.	Normal.		Normal		All (accidentally killed before hatching)	
11	1st. pole body forming	5 min.	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{2}$	Incomplete	Normal		Normal		All	0
12	1st pole body forming	3½ min.					Normal		Normal		All (probably)	
13	1st pole body formed	5 min.		$\frac{3}{4}$	$\frac{1}{4}$	Distinct	Normal		Normal		All	0
14	Both pole bodies formed; 30 min. before 1st cleavage.	5 min.					Many abnormal	Abnormal	Many abnormal	Abnormal	ca 40	40
15	Both pole bodies formed	22 min.	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{2}$	Distinct	Many abnormal	Abnormal	Many abnormal	Abnormal	8	18

TABLE 2—continued
Results of centrifuging eggs of Physa ancillaria, Say.

EXP. NO.	CENTRIFUGED		STRATIFICATION OF SUBSTANCES			CLEARAGES		GASTRULAE		YOUNG SNAILS		
	Stage	Duration	Gray	Clear	Yell'w	Degree	Form	Subs.	Form	Subs.	Normal	Abnormal
16	Both pole bodies formed	12 min.		$\frac{2}{3}$	$\frac{1}{3}$		Abn ormal		Abnormal		4	30
17	Both pole bodies formed	12 min.				Distinct	Abnormal		Abnormal		3	30
18	Before 1st cleavage	2 min.				Indistinct					22	60
19	Just before 1st cleavage	5 min.		$\frac{7}{8}$	$\frac{1}{8}$		Generally abn ormal		Generally abnormal		17	29
20	Germ. vesicle to 1st cleavage	4 hrs. slow				Com- plete	Abnormal		Abnormal		0	All
21	2-cell stage	10 min.					Abn ormal		Abnormal		0	All
22	2-cell stage	10 min.					Abn ormal		Abnormal		0	All

is probably dissolved, while a finely granular yellow material gathers around the maturation spindle, the germ nuclei and the segmentation spindle.

Before maturation the gray substance is small in quantity and finely granular, and it is readily separated from the clear substance; after maturation it becomes more abundant, more coarsely granular or alveolar, and it is separated with greater difficulty from the clear substance.

The clear material may be sharply separated from the yellow and the gray before the maturation division; afterward it is separated with greater difficulty, and the amount of it which may be separated decreases up to the first cleavage. It is especially difficult to separate the clear from the gray in the later stages of this period. This may be due to increasing viscosity of the egg substances in the later stages. In many cases it is known that the egg substance increases in viscosity after maturation, probably through the loss of water. Many observers have noted the loss of water on the part of the egg immediately following the maturation and fertilization. In *Fulgur* (Conklin 1907) this change in the consistency of the oöplasm is most notable, the eggs being quite fluid in the early stages and much firmer in the later ones.

2. The changes in the relative weights of the three oöplasmic substances is shown by the rapidity with which the centrifuged eggs orient at different stages. Until the first maturation the centrifuged eggs orient very rapidly, with the yellow pole down and the gray pole up. After maturation the stratification of the substances becomes less and less distinct and at the same time the eggs orient more slowly until at the time of the first cleavage this orientation is so slow that it usually escapes detection. The same is true of eggs centrifuged after the maturation and before the first cleavage; such eggs always orient the more slowly the later in this period they are centrifuged. This change in the rate of orientation may be due in part to a less distinct separation of the three substances in the latter part of this period, owing to the greater viscosity of the egg substances. But that it is due in large part to changes in the specific weights of the substances

themselves is shown by those cases in which by long or hard centrifuging the substances were very completely separated in stages just before the first cleavage. Such eggs also orient slowly, though the yellow and gray zones are sharply separated from the clear one. The progressive changes in the qualities of the substances at the two poles of the egg have already been mentioned, the gray substance passing from a finely granular to a coarsely granular condition, and the yellow substance from a coarsely granular to a finely granular state.

On the whole, then, several important physical changes take place in the substances of these eggs between the periods of maturation and cleavage; these changes affect the quantities and qualities of the different substances, as well as their relative weights and viscosities.

3. As has been shown (p. 420) the germinal vesicle at the time of laying is slightly eccentric toward the future animal pole of the egg, but when the eggs are centrifuged in this stage they lie in all possible positions and consequently the axis, of stratification may bear any relation to the chief axis of the egg. Consequently this stratification may take place in the chief axis, at right angles to it or anywhere between these two extremes. Nevertheless in eggs centrifuged before the formation of the first maturation spindle, the polar bodies always lie on the clear zone or adjoining it on the borders of the yellow or gray zones. A study of such eggs at the time of maturation shows that the spindle lies in the clear zone and is apparently prevented from moving into the gray or yellow zones. In all cases then in which the axis of stratification does not coincide with the maturation axis the latter has been shifted so as to cause the polar bodies to lie on the clear zone. Such eggs almost invariably develop normally, as a glance at table 1, Exp. 1-4, will show, but in such cases the polar bodies do not always lie at the animal pole. It seems probable therefore that while the maturation axis has been altered by centrifuging the chief axis of the egg has remained unchanged.

When eggs are centrifuged during the first maturation division, the polar bodies invariably form on the gray zone, though in some cases (Exp. 5) near the edge of this zone. Either the entire egg at

this stage orients so that the animal pole is directed toward the center of the centrifuge, or if the eggs do not orient on the centrifuge, the maturation spindles must be shifted so that they come to lie in the axis of stratification. I have not been able to determine with certainty which of these possibilities is actually realized in these eggs, but in *Crepidula*, as I shall show in another paper, it is almost impossible to move the spindles by centrifugal force; it seems probable therefore that in this case also the spindles do not move, but that the eggs orient, so that their chief axes come to lie in the axis of stratification.

After both maturation divisions the stratification may take place in any axis; consequently the polar bodies may lie on any zone. However in all cases the egg retains or recovers its original polarity after its removal from the centrifuge. I conclude therefore that the chief axis of the egg is fixed at all stages and may not be altered by the shifting of egg substances, or maturation spindles, or the point of extrusion of polar bodies. This conclusion is in substantial agreement with the work of Lillie (1909) and Morgan (1909).

4. As the position of the maturation spindle under normal conditions proclaims the polar differentiation and the chief axis of the egg, so the position of the first cleavage spindle in normal eggs announces the plane of bilateral symmetry. This spindle lies in the plane of symmetry and the first cleavage separates an anterior from a posterior blastomere. The oöplasmic substances may be shifted in any direction with regard to this plane of symmetry, usually without changing the plane itself, and since this may occur before the cleavage spindle is formed it is evident that the position of the spindle is the result rather than the cause of bilaterality. The same is true of the positions of the egg and sperm nuclei and of the cleavage centrosomes.

In some cases the cleavage spindle may be moved in position so that the first two blastomeres are unequal in size (fig. 32) and yet normal development may result. There is little evidence however that the cleavage spindle can be turned from its position at right angles to the chief axis, or that the cleavage furrow can be made to form an angle with that chief axis. But while the nucleus

and especially the spindle is displaced with difficulty, by centrifugal force, it seems probable from many observations that its position in the cell is determined by something within the cell body. This has been shown to be the case in *Crepidula* (Conklin, 1902), where the future position and direction of the spindles are, in some cases, proclaimed by the form of the cytoplasm before the spindles are formed.

In 1903 I suggested that the inversion of the symmetry of sinistral, as compared with dextral snails, might be due to an inversion of the polarity of the egg at the time of maturation. I had hoped that by centrifuging the eggs of dextral and of sinistral snails at very early stages I might be able to artificially invert their symmetry. However, this hope has not been realized except in a few doubtful cases. In a few instances there developed from centrifuged eggs small snails with wide-mouthed conical shells which usually showed no spiral twist; and in one case (Exp. 18) two small snails developed, but did not hatch, in which the apex of the cone-shaped shell was turned forward and to the right, whereas in the normal snails of this species (*Physa ancillaria*) the apex of the shell turns backward and to the left. In one of these two snails the heart, which was easily recognized by its beating, was on the mid-dorsal line, in the other (fig. 46) it was on the left side, whereas in normal specimens it is on the right. This represented a partial inversion of symmetry, at least, but since such inversion occurred only twice among hundreds of centrifuged eggs, and since it never occurred in isolated eggs, the history of which was known from the beginning, it cannot be affirmed that it was produced by an inversion of polarity at the time of centrifuging, and its precise cause remains unknown.

In the paper referred to I mentioned the fact that the polar bodies in *Crepidula* might be caused to be extruded at the vegetative pole, and I assumed that the polarity of the egg might thus be inverted. I did not then know that the final polarity of this egg might be unchanged, irrespective of the position which the polar spindles and the polar bodies may be forced to assume. In view of the fact that it is quite impossible by the methods hitherto employed to invert the polarity of the egg, it is evident that the

symmetry cannot be inverted by these methods. The evidence seems to me more than ever conclusive that inverse symmetry is associated with inverse polarity, but polarity is evidently a more fixed and fundamental property than I had formerly supposed.

5. It is very evident that the injurious effects of centrifuging increase rapidly from the time of the first maturation to that of the first cleavage. Before the formation of the first polar body the eggs stand centrifuging without much injury. After both polar bodies have been formed and up to the period of the first cleavage the effects of centrifuging are more and more injurious. Experiments 1-5 and 10-12 show that when eggs are centrifuged for about 10 min. in the germinal vesicle stage they generally develop normally; when centrifuged for a like time during the formation of the first and second polar bodies, about half of the eggs develop normally, while the other half become abnormal (Exps. 6, 7, 14); when centrifuged after both polar bodies are formed a much larger number develop abnormally until, in the case of eggs centrifuged at the time of the first cleavage, every egg becomes abnormal (Exps. 7-9 and 15-22). It is evident that these injurious effects are not due to mere separation of oöplasmic substance, nor to the axis of such separation, for the effects of centrifuging are least injurious precisely at the time when the substances can be separated most completely and in any axis of the egg. It is also evident that the injurious effects of centrifuging are not due primarily to injuries to the mitotic figure, since these injuries are not limited to eggs centrifuged during periods of mitosis. Eggs centrifuged during the maturation divisions usually develop normally, those centrifuged in the resting stage before the first cleavage rarely do. The injurious effects of centrifuging increase gradually from the period of the first maturation to that of the first cleavage irrespective of whether the eggs are centrifuged when in division or in rest.

It seems probable that this increase in the injurious effects of centrifuging as the egg approaches the first cleavage stage is due to (a) increasing differentiation of the egg, and (b) decreasing opportunities for readjustment of displaced substances. In all

cases in which the three substances have been sharply separate the clear protoplasm afterward diffuses slowly into the gray and yellow zones, and if some time elapses between the centrifuging and the first cleavage, the clear zone may be largely diffused through the gray and yellow ones before the new division wall forms; but when centrifuging occurs just before the cleavage this readjustment does not take place so fully.¹

On the other hand it is very probable that there is an increasing differentiation of the oöplasm between the maturation and the first cleavage. This is indicated by the fact, among others, that all experiments on the cleavage stages of gasteropods indicate that as early as the first cleavage differentiation has progressed so far that the development of isolated blastomeres is strictly partial, (Crampton 1896). That there is a progressive differentiation of the cytoplasm in the period between maturation and cleavage is shown also by the work of Wilson (1903) and Yatsu (1904) on *Cerebratulus*. I have not found it possible to apply the methods used by these investigators to the study of the eggs of these pulmonates, protected as they are by resistant, elastic capsules, but the results of my experiments with centrifugal force are in full accord with the works mentioned, and these results find a ready explanation if there is a progressive increase in the differentiation of the egg substance.

In conclusion then, it seems probable that the injurious effects of centrifuging, in the latter part of the period before cleavage, are due principally to the greater differentiation of the oöplasm at this period. This conclusion suggests a possible means of harmonizing the discordant results so far obtained in centrifuging the eggs of different animals, as for example, those of echinoderms and ascidians. If the injurious effect of centrifuging is proportional to the degree of differentiation of the oöplasm, these apparently discordant results may find a ready explanation.

6. Finally these experiments show that the differently colored substances of these eggs are not "organ forming" in the sense

¹ In eggs which have been killed by centrifuging, such diffusion of the clear material into the yellow and gray zones does not take place, but the three zones remain distinct indefinitely.

that each can give rise to only one organ or set of organs. In the normal development the clear and gray substances are largely contained in the micromeres, or ectomeres, the yellow substance in the mesomeres and entomeres. But in the centrifuged eggs the stratification of these substances may take place in any axis, thus locating the yellow material, for example, in the dorsal, the ventral, the anterior or posterior, the right or the left halves of the body, and yet the form of development may be perfectly normal in every case and young snails may hatch from such eggs and live indefinitely. Indeed the gray material may be thrown entirely out of the egg without interfering in the least with its normal development (figs. 27-30).

In the study of these eggs one cannot avoid the impression that both the gray and the yellow substances are mere inclusions in the protoplasm and that neither are essential to development (*vide* Lillie 1906, 1909; Morgan 1908-1909.) The clear substance, on the other hand, seems to be the real protoplasm of the egg in which the heavier and lighter inclusions are contained, and when these inclusions are separated from the protoplasm by centrifugal force the latter is still capable of typical development. That the clear substance alone is protoplasm is indicated by the fact that it alone of the oöplasmic substances increases in quantity during development, and it alone contributes to the growth of the nucleus. The fact that the yellow substance decreases in quantity at the same time that the clear and gray increase is evidence that the former is converted into the latter; and in those centrifuged eggs in which almost all of the yellow material is contained in one of the first two blastomeres and the clear and gray substances in the other it may be seen that clear substance ultimately appears in the yellow cell. Evidently a small amount of clear substance may have been left in this cell, but just as evidently clear substance must be formed anew. This may take place in all cases in which a nucleus is left in a cell and consequently the nucleus must have power to form hyaline protoplasm or to transform yolk into protoplasm.

But if the clear substance is protoplasm this work gives no evidence that it is so differentiated that certain portions of it are

destined to give rise to certain organs, and that they are incapable of forming anything else. Apparently differentiation does not reach this stage before cleavage in these eggs. The differentiations of polarity and symmetry are, however, established before cleavage begins. But it is evident that polarity and symmetry do not reside in the clear substance alone from the fact that the distribution of the clear substance with respect to the chief axis of the egg, or with respect to the plane of symmetry may be almost as varied as in the case of the yellow and gray materials and yet normal development may result. If there be a ground substance in the sense of Lillie (1906) in which polarity and bilaterality inhere, it should presumably be identified with the clear protoplasm and polarity and symmetry should be permanently altered when the mass of the clear substance is displaced. This is not always the case; most of the clear substance may go into one cell, the yellow into another and yet the final polarity and bilaterality of the developing embryo may not be changed.

I cannot find therefore that either polarity or bilaterality is unalterably associated with any one of the three visible substances of the egg. On the other hand it may possibly reside in some tenuous framework which interpenetrates the entire cell and which is not disturbed by the shifting of the visible substances of the egg. I can offer no direct evidence for the existence of such a framework, distinct from the mass of hyaline protoplasm. Nevertheless it seems necessary to assume that there is some permanent organization which is not destroyed by centrifuging, and which may be able to bring back to their normal positions displaced nuclei and cytoplasmic substances. When most of the hyaline protoplasm goes into one of the first two blastomeres and most of the yolk into the other, such a return of displaced substances to their normal positions is impossible after the division wall between the two cells has been formed; but if there is a nucleus in the yolk cell, the cytoplasm of this cell may gradually increase in amount until approximately normal relations are restored. While therefore it is possible for the visible polarity or bilaterality of an egg to be changed experimentally, there is apparently something which does not change, and which is able to restore, by a process of regulation, the original organization.

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DESCRIPTION OF FIGURES

All figures are those of the living eggs or embryos of fresh-water pulmonates, and most of them are freehand drawings.

1-22 Normal development of the eggs of *Physa* and *Planorbis*, showing the normal distribution of the yellow and clear substances during the maturation, fertilization and early cleavages of the egg. The yellow substance is ultimately confined very largely to the entomeres and mesomeres at the vegetal pole.

23-26 Successive stages in the development of an egg of *Lymnaea* centrifuged in the germinal vesicle stage. The nucleus lies in the clear zone and in this zone, the first maturation spindle forms. During the formation of the first polar body, which always lies on the clear zone, the egg constricts in the region of this zone, but this constriction never leads to complete division.

27-30 Successive stages in the development of an egg of *Lymnaea* centrifuged during the second maturation division. A deep constriction appears through the clear zone, which often leads, as in this case, to the complete separation of the gray sphere. This gray sphere is really the second polar body to which the normal first polar body is attached, and it undergoes no further development. The yellow part of the egg gives rise to a normal embryo and adult.

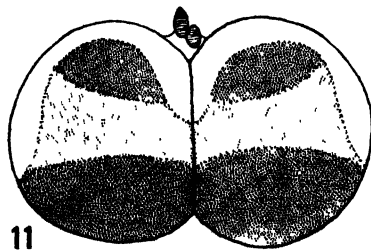
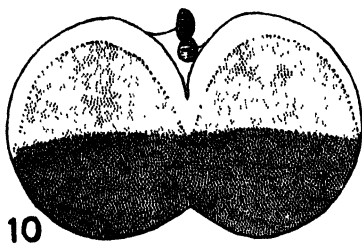
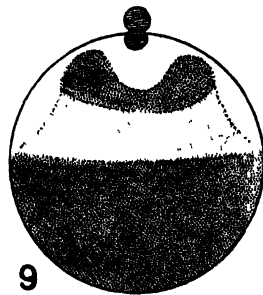
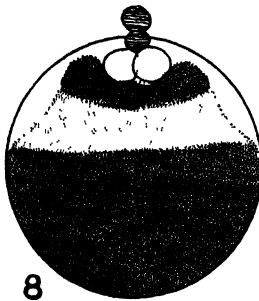
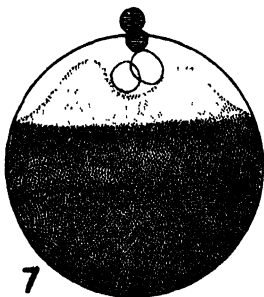
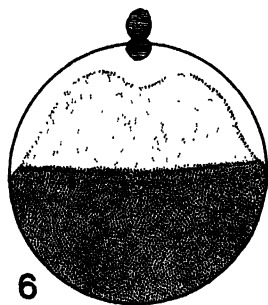
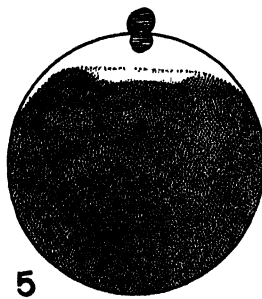
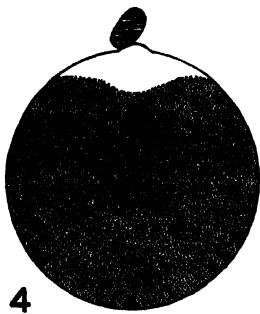
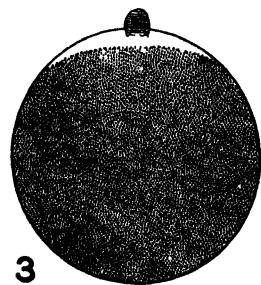
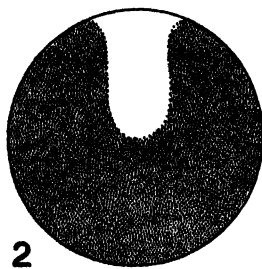
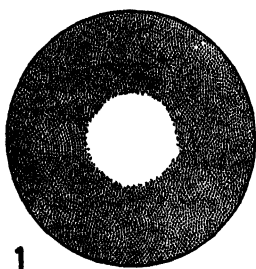
31-33 Successive stages in the development of an egg of *Physa* centrifuged 22 min. after the maturation divisions. The eggs were drawn out into cylinders (fig. 31), and in many cases the first cleavage was unequal, the small cell being yellow and the large one clear. Such eggs are capable of normal development.

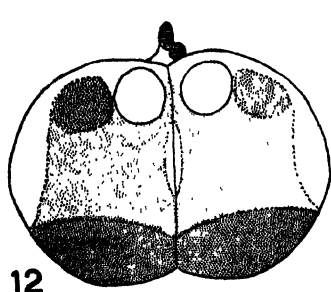
34-37 Successive stages in the development of an egg of *Lymnaea* centrifuged after maturation, in which one of the first two blastomeres is chiefly gray, the other chiefly yellow. In the rotating embryo (fig. 37) the gray substance is largely located in the anterior half, the yellow in the posterior half.

38-41 Stages in the development of an egg of *Lymnaea* centrifuged after maturation, in which the first two blastomeres have an equal portion of the three substances, the axis of stratification being in the plane of the first cleavage. In the rotating embryo (fig. 41) the yellow substance is largely located in the right half of the body, the gray in the left.

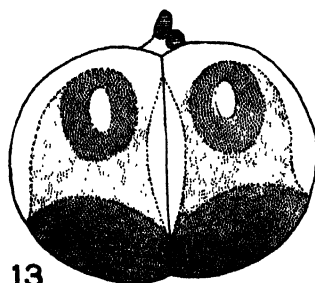
42-45 Stages in the development of an egg of *Lymnaea* in which the axis of stratification is oblique to the first cleavage plane, and in which the distribution of substances in the embryo (fig. 45) is oblique to the chief longitudinal and cross axes.

46, 47 Young snails of the genus *Physa*, which have developed from eggs similar to those shown in figs. 31-33. In fig. 46 there is a partial inversion of symmetry, the apex of the shell coiling forward and to the right, whereas in normal cases it coils backward and to the left. The heart also is transposed in position. In fig. 47 the young snail is normal, except that the whole buccal region, together with the radula is everted. This is a rather common abnormality in these centrifuged eggs.

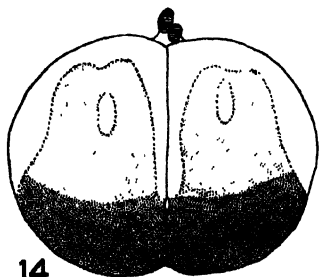




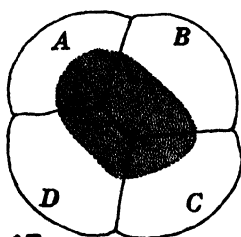
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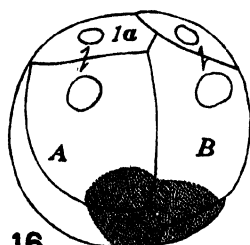
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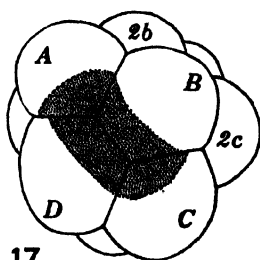
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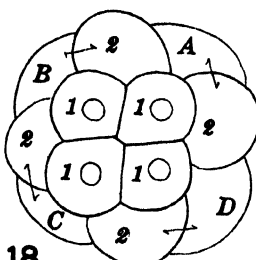
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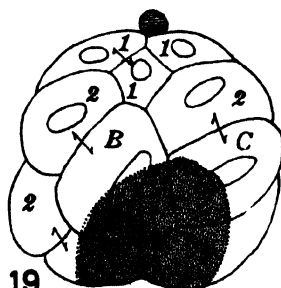
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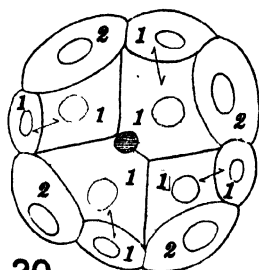
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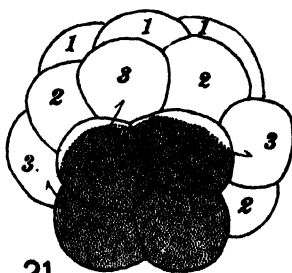
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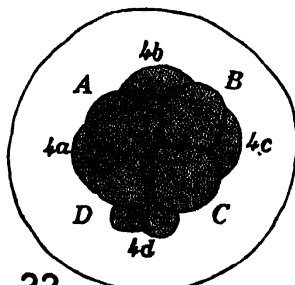
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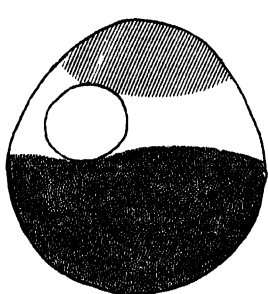


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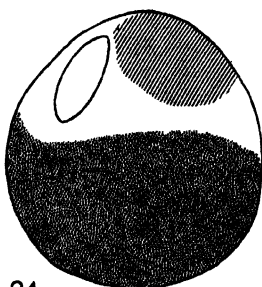


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EFFECTS ON EGGS OF FRESH WATER PULMONATES



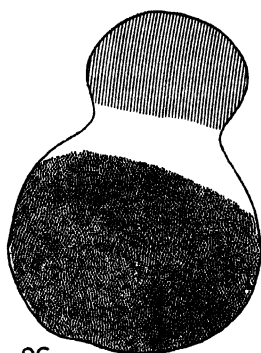
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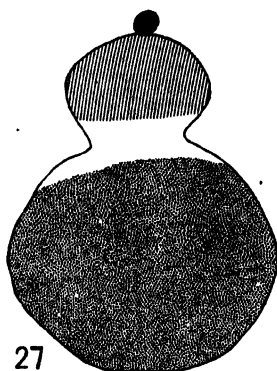
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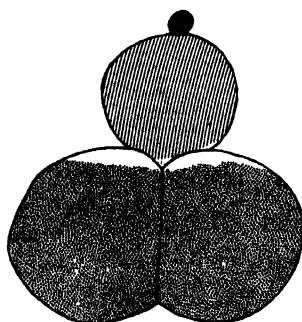
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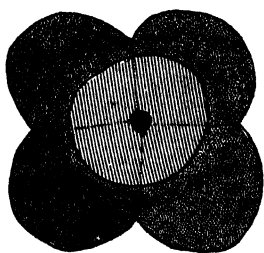
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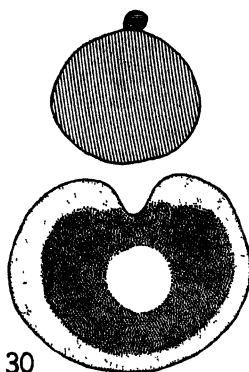
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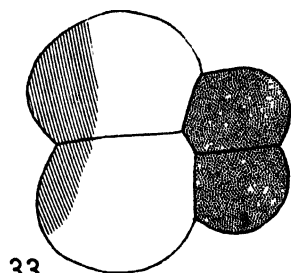
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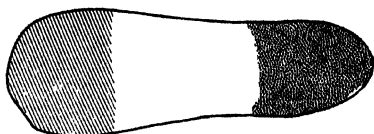
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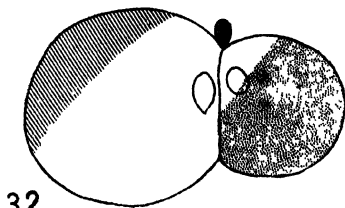
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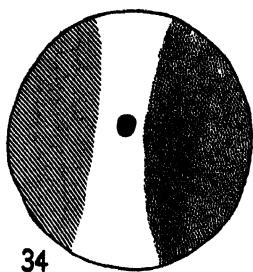
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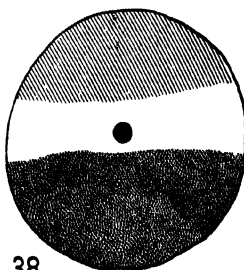
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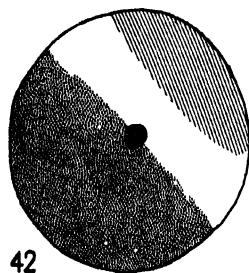
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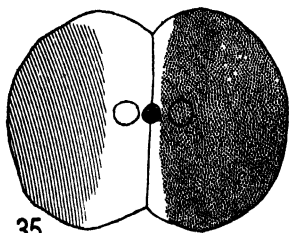
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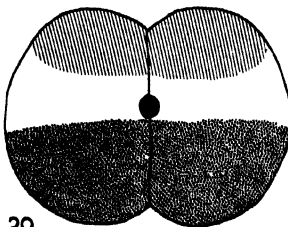
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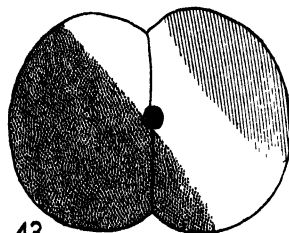
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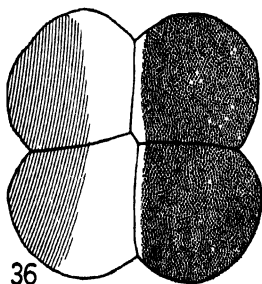
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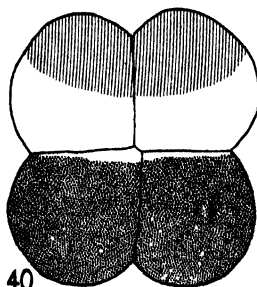
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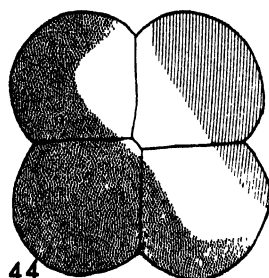
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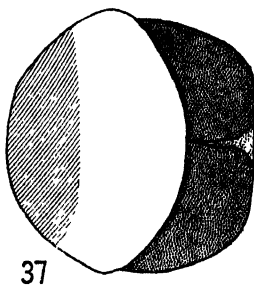
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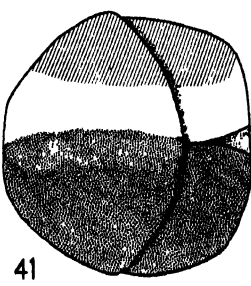
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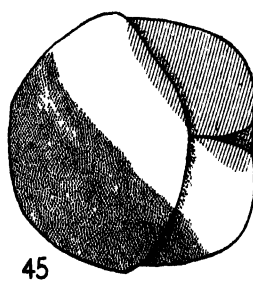
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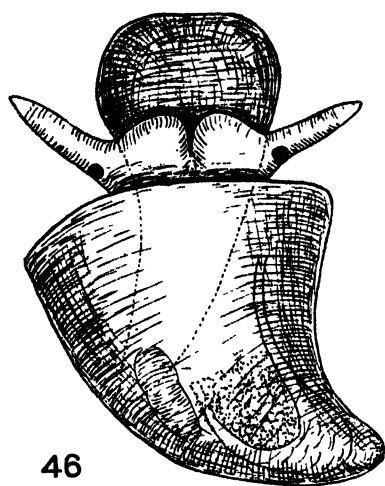
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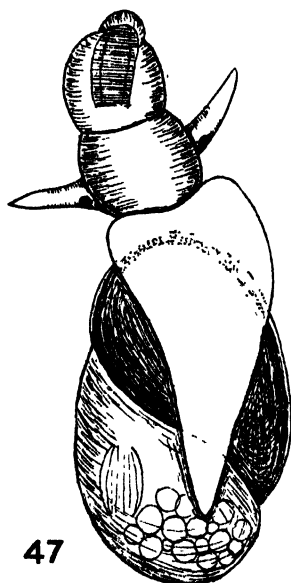
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THE PRIMITIVE PORES OF POLYODON SPATHULA (WALBAUM)

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Minnesota*

TWELVE FIGURES

Polyodon spathula, popularly called Spoonbill and Paddlefish, is one of the most individualistic and interesting representatives of the living Ganoids. Among the striking external features are hundreds of groups of black spots on both the dorsal and ventral surface (excepting a narrow area along the mid-dorsal and mid-ventral line) of the bill. The spots of a group may be arranged in two to five, or more, clearly marked smaller, primary groups of two to ten individual dots. Now and then a single spot is located quite apart from others. As a rule the spots are grouped in the area corresponding to or lying over the meshes of the bony network of the bill. They rarely lie immediately over a plate of bone. Figs. 1, 2, 3, 4 will give a better idea of the arrangement and distribution of these spots on the bill of *Polyodon* than can be gained from a description. Similar groups of spots are found on the outer surface of the operculum, about the eye and other regions of the head. Their distribution on the operculum and side of the head is shown in fig. 5. A large group may contain as many as sixty dots, but as a rule a group contains less than half that number, particularly on the bill. The largest groups have been found on the operculum. There is, however, considerable insignificant variation among the individuals in both the size of the groups and their distribution on the operculum and other parts of the head. On the bill the size of the groups of spots and their distribution are more or less determined by the bony network of the bill. Figs. 1 and 2 show their distribution on a large



Figs. 1 and 2. Photographs of the dorsal and ventral surface respectively of a bill 300 mm. long from the tip of the bill to the eye. The fish was a little more than one meter long from the tip of the bill to the tip of the dorsal lobe of the tail. The clusters of lateral line branches shown in fig. 2 were painted white to make them stand out more clearly. As a rule they are inconspicuous. Fig. 2 was inadvertently photographed on a slightly larger scale than was fig. 1.

Figs. 3 and 4. Photographs of the two halves of a portion of a bill cleared in caustic potash. The black lines represent the sheaths of pigment cells surrounding the blood vessels. The groups of black spots represent the groups of primitive pores. In fig. 4 the lines and spots were accentuated with India ink. Fig. 3 has not been retouched.

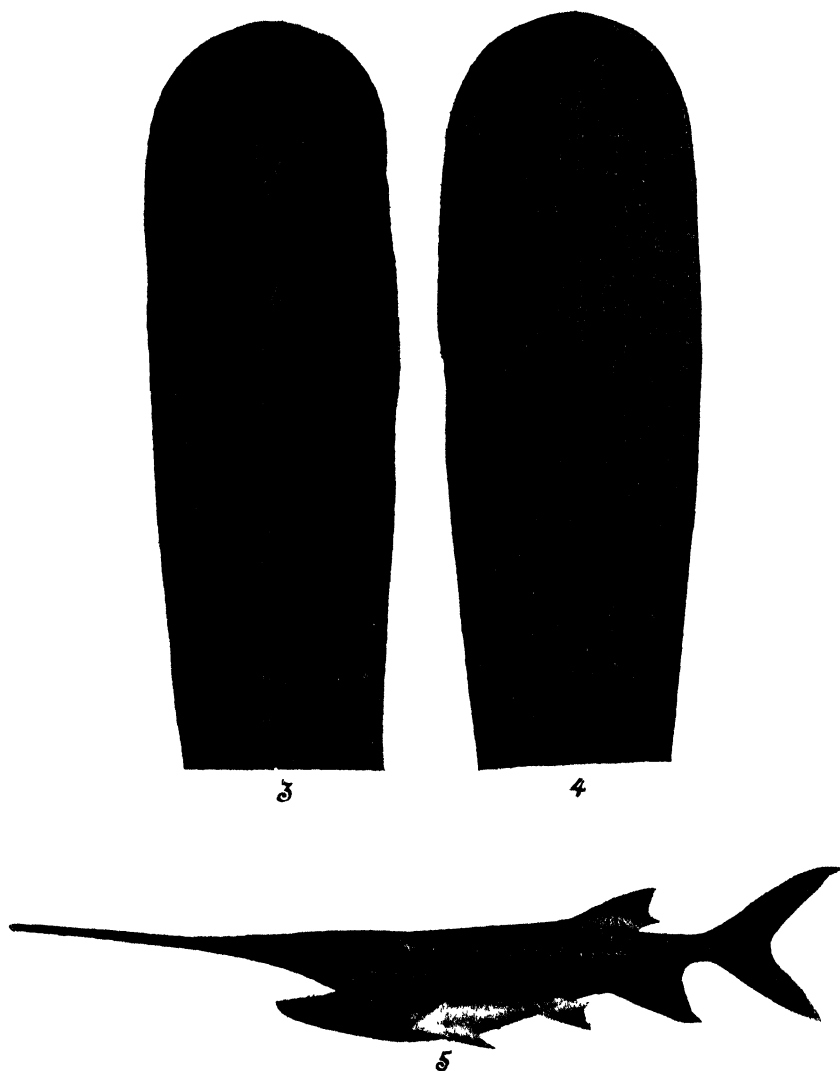
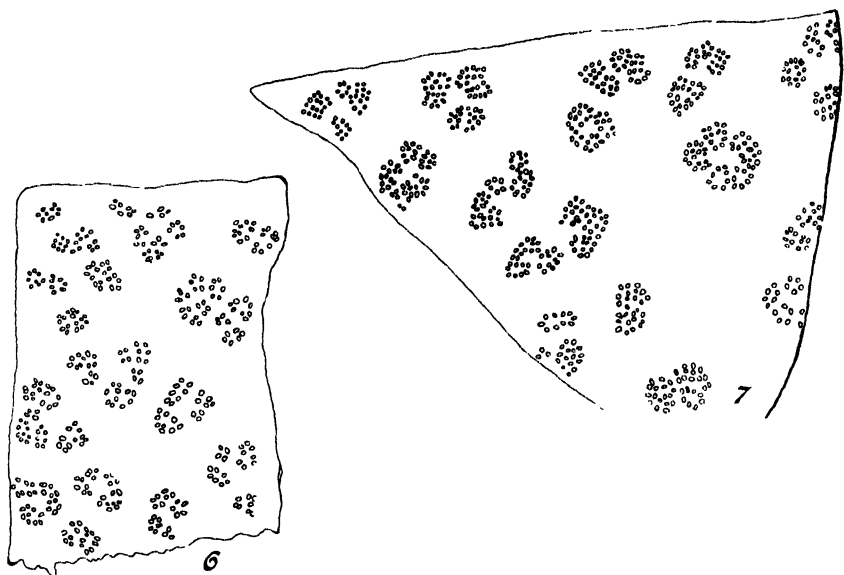


Fig. 5. Photograph of a *Polyodon spathula* on which the lateral line and its branches and the hyomandibular branch and its branchlets had been painted white. Many of the groups of primitive pores on the side of the head were inked to make them stand out more clearly. The slight humping at the dorsal fin is due to the way the fish was suspended. The fish measured about one meter from the tip of the bill to the tip of the dorsal lobe of the tail.

bill Figs. 6 and 7 show in detail the grouping in a small area respectively on an operculum and the dorsal surface of a bill.

Careful examination discloses the fact that each dot represents at least one small pit surrounded by pigment cells, which to the unaided eye appear as one black mass. Toward the free edge of



Figs. 6 and 7. Camera lucida sketches showing the grouping of the primitive pores respectively in a small area on the ventral surface of a bill and on the end of an opercular flap. Drawn by Helen A. Sanborn.

the operculum the pigment cells often are less abundant about the pits than they are elsewhere. In some of the groups of pits the pigment cells are almost wholly absent, and in such places the pits can be located only by close inspection. All the pits here noted were called primitive pores by Collinge,¹ and they will be so termed in this article. For convenience I shall also refer to them as pits. There are from fifty to seventy-five thousand of these pits on the bill, head and operculum of *Polyodon*.

¹ The Sensory Canal System of Fishes. Part I.—Ganoidei. By Walter Edward Collinge. *Q. J. Mic. Science*, vol. 36, New Series, 1894.

The primitive pores are simple epithelial invaginations about two hundred microns deep and about seventy-five to one hundred microns in transverse diameter. Definite measurements are given of several pits in connection with fig. 9. The primitive pores usually contain some mucous and not infrequently they are full of mucous. The mouth or opening is usually smaller than the diameter of the pit near its bottom. As a rule each pit has its own opening or mouth, but not infrequently two pits, and occasionally three, have a common opening. In form the pit varies from a cylindrical invagination with an almost flat bottom to a more or less pronounced flask-shaped depression with a more or less concave bottom. None of the pits have what might be called a distinct neck. Indeed, as will appear later on, each pit presents just two distinct regions—the differentiated bottom and the undifferentiated region above the bottom. Figs. 8 and 9 show groups of primitive pores respectively in transverse and longitudinal section.

As intimated above each primitive pore is more or less completely surrounded by branched pigment cells, which are abundant in nearly all parts of the body. Fig. 8 gives some idea of the distribution of pigment cells among the pits near the bottom of the pits. Lymph sinuses, blood vessels and nerves are abundant in the subcutaneous connective tissue. Figs. 3 and 4 show the distribution of the blood vessels in the neighborhood of the primitive pores of the bill. The vessels are surrounded by a more or less dense network of branched pigment cells and consequently stand out as black lines. Nerves usually accompany these blood vessels but I have never seen a nerve going to the bottom of a primitive pore.

On the ventral surface of the bill there is a double row of groups of pores that open into small canals connected with branches of the lateral line. These groups are well shown in fig. 2. As a rule they are not very evident, but on some bills they are quite conspicuous. A comparison of the branchlets of the lateral line, shown in fig. 5, with these rather striking structures of the bill, shown in fig. 2, will at once suggest that the clusters of pores and tubes on the ventral surface of the bill represent close groups

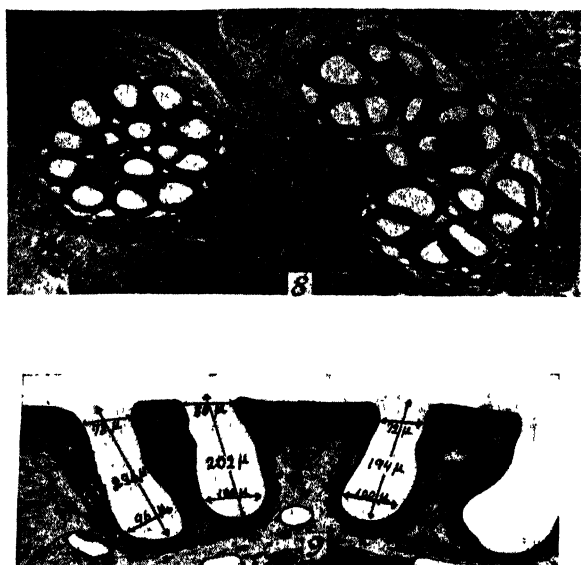


Fig. 8. Photographs of a horizontal or transverse section of several groups of primitive pores showing the distribution of the pigment cells, the lymph spaces and blood vessels among the primitive pores. The section passed through the pores a little above the plane of the floor of most of the pits. The photograph was partially inked to bring out certain features more clearly than others. Photographs with a Zeiss aa, plate about six feet from the object. Figure one-half the size of the photograph.

Fig. 9. Photograph of a longitudinal section of four primitive pores showing the general form of the pits. All the blood vessels, excepting the one next to the number of the figure are empty. A small nerve is shown at the left of the number. The photograph was partially inked, and the reproduction here is one-half the size of the original.

The dimensions of the pits were determined with a Zeiss Screw Stage Micrometer. The photograph was made with a Zeiss C objective, the plate about five feet from the slide.

of branchlets like those of the lateral line. I have never found any of the branches or branchlets of the lateral line system in any way in communication with a primitive pore. The primitive pores are not and, I believe, never have been in communication with the lateral line system. Incidentally it may be remarked that the lateral line of the *Polyodon* shown in fig. 5 is typical, and effectively

corrects some of the erroneous conclusions and figures Collinge based upon his poorly preserved material.

Collinge described and figured the primitive pores as distinct sense organs, each having a definite nerve. The material upon which his description and accompanying figures were based was admittedly poorly preserved, but imagination and a preconception of the structure of a sense organ supplied what the material lacked. The only thing in Collinge's figures that corresponds to fact is the invagination, and even this is not altogether correct. These figures offer an excellent illustration of the danger of basing any description and figures of histological structure upon poorly preserved material.

In 1905, during the session of the American Society of Zoölogists at Ann Arbor, I pointed out Collinge's errors and gave some evidence discrediting the generally accepted view that the primitive pores are sense organs.

In 1906 Kistler² published a paper in which the primitive pores are presented as distinct sense organs. The floor of the primitive pore is described and figured as lined by a single layer of two types of cell—one a clear "supporting cell" and the other a darker, club-shaped "sensory cell" with a definite "slender conical process" on its free surface and a distinct innervation. Fig. 12 is a free copy of Kistler's fig. 6, which illustrates his two types of cells. The nerve fibrils have been omitted.

In 1907 I presented a brief paper on the lateral line system of *Polyodon* at the VIIth International Zoölogical Congress. In this paper I concluded my remarks on the primitive pores with the following statement: "The abundance of the peculiar mucous-like secretion found in many specimens, and the character of the epithelium, indicate at least that the function is not primarily that of a special sense organ. If the primitive pore is a sense organ at all it is one of low differentiation." The figures accompanying the above, while sufficient for general purposes, are not correct in the details of the epithelium. The following

² The Primitive Pores of *Polyodon spathula*. By Herbert D. Kistler, B.S. M.D. *Jour. Comp. Neur. Psych.*, vol. xvi, 1906.

account and figures are based upon recently made preparations that I believe present conclusive and irrefutable evidence against the generally accepted view that the primitive pores are distinctive sense organs.

My best material was obtained from the living fish, fresh from the water. Pieces of the bill, operculum and other parts of the head were immediately placed into various hardening fluids. Since the fish has little vitality out of the water in the summer, and the epithelium at this time of the year sloughs off very easily shortly after the death of the fish, prompt hardening of the material is important. Various hardening fluids, such as formalin, formol-corrosive sublimate-acetic acid, trichloroacetic acid, Zenker and Gilson fluids were used. Small pieces of the hardened material were imbedded in paraffin, cut into series of sections, three, four, five, seven and ten microns thick, fixed to the slide, variously stained and finally mounted in xylol damar. Some pieces were stained in toto and yielded good general results. The connective tissue is apt to become very tough during the processes of hardening and imbedding. It is therefore advisable to limit the material to be cut to the epithelium as far as possible. Sections stained with Heidenhain's iron hematoxylin yielded the clearest positive results and, unless specifically stated otherwise, the following descriptions are based on sections stained by this method. Sections stained with other aqueous, and alcoholic stains, present the same general characters, though not always with the same distinctness.

In sections parallel to the long axis of the primitive pore the epithelium of the bottom of the pit is clearly and sharply marked off from the epithelium lining the rest of the pit. While the epithelium on the floor of the pit is the immediate bone of contention, it is necessary to consider briefly the skin epithelium between the pores and to examine carefully its relation to that of the pore, especially to that lining the floor of the pit.

Just beyond the mouth of the primitive pore the skin epithelium may be ten to fifteen or more cells deep. The outer or surface cells are thin plates. The basal cells are more or less slender columnar cells. In some regions the basal cells are very long and

slender. Whatever the shape of the basal cell, the nucleus lies in the distal region of the cell. The layers between these two extremes present the usual intermediate cell-forms of a many-layered epithelium. In well stained preparations all the cells, excepting the outermost flat cells, have a blue to blue-gray tint. The nuclei stain more deeply in spots but appear quite clear and present a rather open chromatin network in which one can usually recognize a distinct nucleolus, using the term in a morphological sense only. The cells of the outer two or three layers stain more deeply than the rest, appear much denser and have dense, deeply stained nuclei, which appear like slender rods. This difference in structure and affinity for stains makes the outer layers, when seen under a moderate power, appear like a limiting membrane. Just within the mouth of the pit this apparent limiting membrane disappears, the number of layers of cells becomes reduced and gradually, though rapidly, falls to two near the bottom of the pit. At and within the mouth of the pit the surface cells frequently present finger-like processes which, as a rule, slant obliquely toward the mouth of the pit. In color and consistency these processes frequently remind one of the surface cells of the skin. They represent not a special structure but merely the loosened overlapping edges of the cells. Near the bottom of the pit these processes are usually wanting, though shorter, more delicate and less deeply stained processes may be present on the cells next to the edge of the differentiated bottom epithelium. Aside from the appearance due to the overlapping of the cells, the thickness and plane of the section, these processes appear to be artifacts due to partial maceration or imperfect hardening or after treatment. They appear in greatest profusion in preparations whose general appearance reminds one of poorly preserved material. In well preserved material, carefully sectioned and carefully mounted, the fingerlike processes are not prominent and not infrequently are absent altogether. On the other hand they are seen in some preparations that otherwise appear well preserved. It is not at all impossible that the conditions within a pit affect the ordinary surface epithelium much as warm water acts on the skin of the dead fish. The mucous and other conditions may cause stagna-

tion and maceration within the pit and thus lead to a lessening of the overlapping edges of the ordinary surface cells. This suggestion occurred to me but recently and I have not had an opportunity of testing it.

Under a medium power, such as a Zeiss D, the epithelium on the floor of the pit presents a line of deeply stained nuclei. The line of demarcation between these deeply stained nuclei and the lighter, less dense nuclei of the cells lining the rest of the pit is sharp. It can be readily recognized in figs. 9 and 11, although it is not as striking here as it is in the sections. At first glance this floor epithelium appears two layered. A careful examination, however, convinces one that it is only one cell deep but composed of two kinds of cells. The more conspicuous and somewhat more abundant cell is a large, relatively clear, columnar cell, less than twice as long as it is wide, with a relatively clear cytoplasm and a deeply stained spherical nucleus containing several nucleolus-like bodies of which three to six may be in view at one focus. This is Kistler's "supporting cell." For reasons that will appear later I shall call it the flagellated cell. The other cell extends out beyond, and more or less covers the outer ends of the contiguous flagellated cells. The cytoplasm of this cell appears more dense; the nucleus is larger, clearer and usually contains but one conspicuous nucleolus-like body. This is Kistler's "sensory cell." I shall call it the cover cell. The two kinds of cells are very easily distinguished by their nuclei. In both kinds of cells the nucleus lies in the outer portion. In nearly all of the preparations the surface of this differentiated epithelium appears faintly ragged or fuzzy as if covered by bits of mucus.

Under a Zeiss 2 mm. apochromatic objective of 1.30 N.A. and a number 4 or 6 compensating eye piece the differences just noted become more marked and new distinctive features become evident in well preserved material stained with Heidenhain's iron hematoxylin. The cover cell, shown in detail in fig. 10, is compressed in the middle so that in optical longitudinal section it appears more or less dumbbell-shaped. The nucleus lies in the expanded distal portion of the cell and in optical section appears triangular because the lower part of it lies in the compressed

region of the cell. It appears clear and has a loose, large-meshed network in which there usually is one large nucleolus-like body. The cytoplasm appears homogeneous, sometimes suggesting longitudinal striation in the basal and compressed region of the cell. The base or proximal end is always expanded and in close contact with the basement membrane. In most of my sections the free surface appears more or less fuzzy. This appearance is due to bits of mucus adhering to very short and variable pseudopodia. My reasons for not considering these processes permanent cilia or sensory hairs are: 1, They are indefinite and variable in shape and length; 2, There are no basal bodies in connection with them; 3, In many preparations the surface of the cover cell is in intimate connection with the mucus in the pit. In sections showing masses of mucus in the pits the strands of mucus are frequently so intimately related to the surface of the cover cell that it is impossible to determine where the cell substance ends and the mucus begins. In such preparations the free surface of the cover cell is uneven and may have relatively wide projections continued uninterruptedly into masses or shreds of mucus far from the surface of the cell. In sections of material hardened in platino-aceto-osmic and stained with iron hematoxylin the mucus in the pit, the cytoplasm of the cover cells and the underlying ground substance of the connective tissue are stained brown, the connective tissue usually being of a lighter shade than that of the cover cells and mucus. In some cases the mucus is continuous with a similarly colored mass occupying the place of a cover cell and extending down to the basement membrane, looking as if the cover cell had become converted into one mass of mucus. The mucus is never seen in such intimate connection with a flagellated cell. In some of my formalin-gold chloride preparations the surface of the cover cell is studded with very distinct long processes, three to five times as large as those noted above. They look like stout cylindrical cilia with rounded free ends to which are usually adhering small bits of mucus. In all of these formalin-gold chloride preparations the cell substance is very badly shrunken and in every respect the sections suggest very poor preservation. In fact whenever the sections

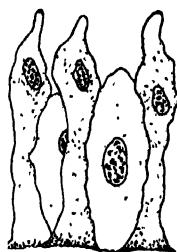
indicate very imperfect killing and hardening or rough after-treatment the processes on the free surface of the cover cell are more pronounced than they are in well preserved material. In some of the preparations many of the cover cells have a relatively clean cut surface, but none of them appear as clean cut as the surface cells of the ordinary lining epithelium. These facts suggested various experiments which I have not had an opportunity of making.

In view of the facts before me I believe the free surface of the cover cell is more or less plastic and can be temporarily projected into more or less pronounced pseudopodia. The position of the nucleus is not that usually occupied by the nuclei of secretory cells. The shape of the cell is also not typical of secretory cells. But the intimate connection between the mucus and the cell compels the inference of an active relationship between the two. The subcutaneous connective tissue is quite slimy—indeed the general appearance and behavior toward reagents of both the nuclei and the ground substance stamp this as a mucoid supporting tissue. And it may be that the cover cells function primarily as the excretors of the mucus formed in the underlying connective tissue.

The flagellated cell, shown in detail in fig. 10, as a rule has a rounded base, the edge resting on the expanded bases of contiguous cover cells. As a rule the base of the cell is slightly shrunken away from the basement membrane. This condition is so common in even apparently perfectly preserved material that these spaces must be looked upon as normal lymph spaces. The flagellated cell differs from all other epithelial cells in that its cytoplasm below and around the lower half of the nucleus contains distinct loosely distributed, granules, which stain more deeply than the rest of the cell substance but not as deeply as the chromatin granules in the nucleus. In sections of platino-aceto-osmic material stained with iron hematoxylin these granules have a deep blue color. They are quite uniform in size and generally appear more or less spherical. In many cells the granules appear to be wanting in a narrow area next to the cell wall, and the cell below the nucleus presents a clear unstained peripheral area sur-



10



12

Fig. 10. Camera lucida drawing of several of the differentiated cells of the floor of a primitive pore. The drawing was outlined on the table with a Zeiss 2 mm. apochromatic objective of 1.30 N.A. and compensating eye piece number 6. Drawn by Helen A. Sanborn.

The second flagellated cell from the right measured 5 microns from the tip of the cone to the basement membrane and about $3\frac{1}{2}$ microns through its largest diameter. The flagellum was barely 2 microns long. The cover cell to the right of this flagellated cell extends about 1 micron beyond the tip of the cone of the flagellated cell. Stained with iron hematoxylin.

Fig. 11. A camera lucida drawing made on the table to show the relations of the differentiated to the undifferentiated epithelium of a pit. The drawing was made after a section stained with Dominici's eosin-orange G-toluidin blue, which does not bring out the flagella. These have been added in accordance with iron hematoxylin preparations. Zeiss 2 mm. apochromatic objective of 1.30 N.A. and compensating eye piece number 6. Drawn by Helen A. Sanborn.



11

Fig. 12. Copied from Kistlers's fig. 6, to show his two types of cells.

rounding a stained and granular inner portion. The free end of the flagellated cell is a short cone in which the cytoplasm is homogeneous and has a faint bluish tint. The apex of the cone is extended as a slender deeply stained flagellum through the opening between the outer ends of the contiguous cover cells. At the base of the flagellum there are two distinct deeply stained basal bodies (diplosome), one below the other and apparently in contact with each other. When I first saw this flagellum I interpreted it as a sensory hair, but the two basal bodies militated against this interpretation. Moreover it was found curved in various directions, which conditions would not obtain if it were a stiff hair. While I have not had an opportunity of verifying this interpretation with observations on living material I do not hesitate in declaring the process a flagellum, because all the morphological features are in accord with it. Having been convinced of the presence of the flagellum in good iron hematoxylin preparations, one now and then gets suggestions of it in sections stained with Ehrlich-Biondi and other stains; but none of the various stains used, excepting the iron hematoxylin, differentiated the flagellum clearly.

A comparison of fig. 12, representing Kistler's two kinds of cells, with my figs. 10 and 11 and the descriptions given above will convince one that there are irreconcilable differences between the two accounts. I can explain these differences only on the assumption that Kistler based his conclusions entirely upon observations made with objectives of too low power.

In the character of the nucleus and the general appearance of the cytoplasm the cover cell is like the cells of the middle and basal layers of the skin epithelium. The flagellated cell, on the other hand, differs from all of them in every respect. Neither kind of cell has the characters of a distinct sensory cell.

The final conclusion accordingly is: Primarily the primitive pore of *Polyodon spathula* is an excretory organ that throws off a peculiar mucus, and the differentiated epithelium of the pore consists of a single layer of two kinds of cells on the floor of the pit—the cover or mucus cell and the flagellated cell.

ON TWO SPECIES OF HYDRACTINIA LIVING IN SYMBIOSIS WITH A HERMIT CRAB

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TWENTY-THREE FIGURES

Two species of Hydractinia have been known among us for many years, which, though very different in external appearance, have the same habit of living always in symbiosis with a hermit crab, *Eupagurus constans* Stimpson, and of forming "shells" of their own entirely composed of a chitinous framework, so that in most specimens there is apparently no basis of gastropod shell, as is the case in most other known species of Hydractinia. The skeleton of one of the species is totally devoid of spines and its substance is very thin and papery, while that of the other is richly armed with large spines, which are conical when small but irregular in shape and branching when large. The latter species has recently been described by Stechow from the Doflein collection and identified by him with the *Hydractinia sodalis* of Stimpson² who in his description of *Eupagurus constans*, men-

¹After writing the above I received a valuable letter from Dr. Stechow, to whom I had written concerning this hydroid, giving some further information in regard to its literature. According to him the hydroid and the hermit-crab are treated on pp. 218-220 of the paper entitled, "Report on the Crustacea collected by the North Pacific Exploring Expedition, 1853-1856," by W. Stimpson, recently published in vol. 49 of the Smithsonian Miscellaneous Collection, 1907, the discovery of the paper being due to Miss Rathbun. According to Dr. Stechow the description in the text and the good figure reproduced in pl. 24 leave no doubt that Stimpson had before him the *Hydractinia* with large spines. For supplying me with this valuable information my best thanks are due to Dr. Stechow.

²Stechow: '07, p. 192; same: '09, p. 21.

tioned a species of *Hydractinia* living in symbiosis with it.³ Since Stimpson's paper is not accessible to me I have no basis on which to form my own opinion, but if, as Stechow says, Stimpson devoted only two lines to the characterization of his *Hydractinia sodalis*, it is very doubtful whether it was precise enough to enable one to decide which of the two species mentioned at the beginning of this paper was before him. Since, however, it is not desirable to introduce unnecessary confusions into zoölogical nomenclature, which threatens to become formidable, I prefer to accept Stechow's identification. The other species is, in my opinion, new, and I propose the name of *Hydractinia spiralis* for it. These two species are the subjects of this paper, and it is but fitting that I should dedicate it to the memory of the lamented Dr. Brooks, since it was from him that I received my first advanced instruction in hydroid morphology.

1. HYDRACTINIA SODALIS STIMPSON

The skeletons of this species are rather common and sold in a dry state at Enoshima and other places, where they are known as "Igaguri-gai," or "chestnut burr shell." I have been told that when Prof. E. S. Morse was here he was interested in these "shells," and was aware that there was always a gastropod shell in the apex. Aside from Stimpson, whose reference to this species has been above mentioned, Inaba is the only author who to my knowledge has described the animal. Owing, however, to the difficult conditions under which he had to work, he regarded it doubtfully as a species of *Podocoryne*. His description is as follows:⁴

36. *Podocoryne* sp.? (Figs. 103, 104, 105.)

Trophosome.—The chitin of the hydrorhiza is very strong and bears at places chitinous spines with branches. Hydranths large, growing out in numbers from a hydrorhizal substratum; there are two forms of hydranths, one large, the larger ones 5 mm. high and 1 mm. across, with about 30 filiform tentacles arranged in a single row

³ Stimpson: '58, p. 225.

⁴ Inaba: '92, p. 96.

near the mouth; the other as long as the former but very slender, with over 60 filiform tentacles arranged in several rows around the mouth.

Gonosome—Unknown.

Locality—Between Misaki and Jogashima;⁵ covering shells containing hermit crabs. It was collected by Mr. I. Shishido in April, 1889; but there are no reproductive bodies. It cannot therefore, be decided whether it belongs to Podocoryne or Hydractinia; but there are two forms of hydranths, and the species has been referred provisionally to Podocoryne on the assumption that the larger polyps are nutritive and the smaller ones give rise to the reproductive bodies. The two forms of hydranths are comparatively large.

The figures accompanying the description leave no doubt that it refers to the species before us, although the entire form of the colony is not reproduced. It is, however, but natural that Inaba's description has not been recognized by western students of hydroids, since it was published in the Japanese language. Therefore Stechow's recent descriptions are practically the first adequate ones accessible to general students. They, but especially the full description of '09, are, as a whole, sufficiently accurate and generally accessible, so that I shall confine myself to one or two remarks and the addition of a few details not mentioned in his descriptions.

First as to its occurrence. It appears to be common in several parts of the Bay of Tokyo and of Sagami, and according to Stimpson it occurs as far north as Hakodate. Stechow,⁶ gives its bathymetrical range as 7-180 m. (4-100 fathoms), and for six of the specimens collected by Haberer it is stated with a query that they were found on the beach—"Strand?" The specimens collected by myself are all from the Bay of Tokyo, off the small village of Kanazawa, where they can be easily obtained by collecting with the hand trawls commonly used by the fishermen of this part. They are from shallow waters, about 16 meters or so, and I have also picked up living specimens on the beach, where they were evidently washed up with the hermit crab still alive and occupying the skeletal shell.

⁵ A small island off the town of Misaki.

⁶ *Loc. cit.*: '09, p. 24.

As to the relation between the gastropod shell and the chitinous skeleton of the hydroid, Stechow thinks that the former is dissolved by the latter. In his full paper he says,⁷ "Bei unserer Form breitet sich die druchweg chitinöse, nicht kalkige, hell—bis dunkelbraune Skelettschicht krustenförmig über die Schnecken-schale aus, die sie offenbar sehr schnell auflöst. Ich fand nirgends, nicht einmal bei jungen Kolonien, auch nur Spuren von Kalk, weder in den Stacheln noch in der eigentlichen Schale." This is, as already suggested, a mistake. It is true that in most cases the larger part of the "shell" is composed entirely of the chitinous skeleton of the hydroid, but if one breaks open its apex a small gastropod shell, *Columbella*⁸ or other genera is invariably found retaining its natural surface intact. It may therefore be inferred that the hydroid colony starts on the surface of a gastropod shell probably already inhabited by *Eupagurus constans*, and that after covering up the whole surface of the shell it grows out beyond its mouth. The hermit crab also growing hand in hand with the hydroid colony, and having no need of migrating into a new and larger shell to accomodate its growing body, there is thus established a permanent symbiosis between the two animals. That this is the case is proved by specimens occasionally met with, in which the hydroid apparently started on a comparatively large shell and has not succeeded in covering up its whole surface but has left a part still more or less exposed. Such a one is shown in fig. 2, in which the shell is a species of *Buccinidæ*, and in which the exposed part is seen to have many small circular holes. That these are, however, not due to the action of the hydroids is very clear from the fact that they are most numerous where the hydroid colony is still very thin. In view of the destructive action of *Hydractinia echinata* (in this case in symbiosis with a *Pagurus*) on the shell which it covers, as was observed by Carter,⁹ it is nevertheless not impossible that our hydroid may have a certain action on the substance of the shell, although such is not apparent in

⁷ *Ibid*: '09, p. 22.

⁸ For the determination of the shells mentioned in this paper I am indebted to my friend, Prof. T. Iwakawa of the Imperial Household Museum.

⁹ Carter: '73, p. 2.

any of the specimens examined by me. There is no doubt that the chitinous spines of the hydroid have no relation to the spines of the gastropod shell, since the latter are usually absent. Again, if, as Stechow supposes, the entire colony is developed on the surface of a gastropod shell which is then dissolved away, the skeleton of the hydroid ought to form an exact cast of the shell; but that the former may depart widely from any form of gastropod shell may be seen on examining a few specimens of the hydroid. This point is also shown very well in the photographic reproductions of Stechow,¹⁰ in which several degrees of spiral winding are manifest.

The outer surface of the hydroid, which is chestnut brown in color, is sculptured by numerous fine, irregular, elongated, anastomosing ridges and furrows, which are especially apparent on the surface of the spines, but are completely hidden from view where the surface is covered by a thick layer of coenosarc. Their appearance and arrangement vary so in different specimens that a general description can hardly be given, the anastomoses in particular being so numerous in some cases that a fine reticulated appearance is brought about. The inner surface of the hydroid shell is perfectly smooth, being lined by a very thin subcontinuous chitinous lamella which would come in direct contact with the body of the hermit crab. The thickness of the chitinous shell may amount to more than 1 mm., the spines being left out of account. In the body of the shell the chitin is comparatively soft, and there is no difficulty in preparing thin free-hand sections with ordinary razors. In such a section carried across the whole thickness of the shell, it is seen to consist of a dense spongework, exactly as in *Solanderia* (*Dendrocoryne*, *Ceratella*, *Dehitella*, *Spongocladium*, *Chitina*) (fig. 3). Toward the outer surface the meshes are larger, the trabeculæ are stouter and darker in color, and are seen to form projections and indentations corresponding to the ridges and furrows on the surface. The meshes become smaller and rounder at a short distance from the outer surface and remain nearly constant inwards. The inner surface

¹⁰ *Loc. cit.*: '09 pl. 1.

is lined by a subcontinuous thin layer of chitin which stains deeply with haematoxylin. In favorable places the trabeculae are seen to consist of superposed lamellae around an axial substance.

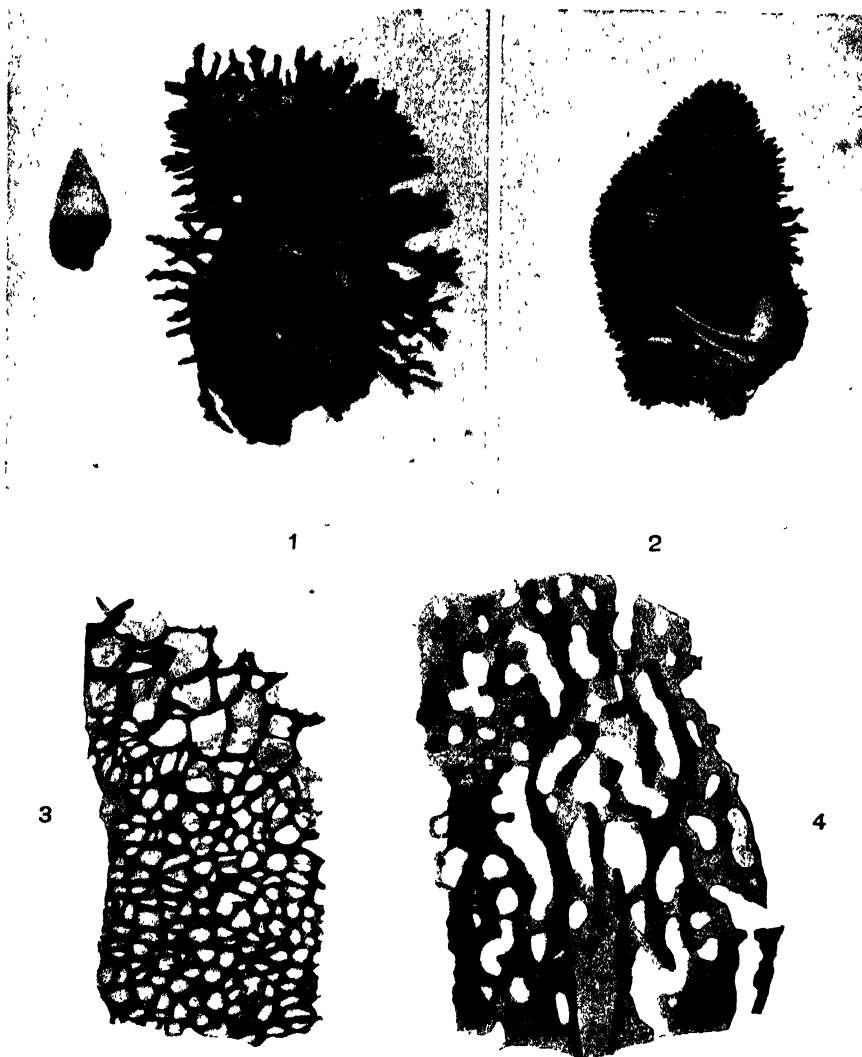
The spines, which form a characteristic feature of this species, are very numerous and of various sizes and shapes from simple, short, rounded conical projections to large, stout branching, antler, like spines nearly 2 cm. in height and 1 cm. or more between the tips of the outermost branches. The inner structure is essentially the same as in the body of the chitinous shell, with the difference that in the spines the meshes are largely elongated and are arranged with their long axes parallel to the length of the spines, so that in a longitudinal section, such as is reproduced in fig. 4, it is common to see long tubular cavities, especially in the axial parts. The formation of the chitinous trabeculae from concentric layers around an axial substance is especially clear in sections of the spines, the axial substance being sometimes very conspicuous by its opacity. Stechow mentions in cross-sections of the spines "zwei deutlich geschlossene Röhre mit etwas dickerer Wandung," which he considers as possibly the first formed part of the spine,¹¹ but according to my observations they are constant neither in number nor occurrence.

As to the mutual relation of the chitinous skeleton and the soft parts, it may be said that it is in general similar to what obtains in *Solanderia*;¹² that is to say, each mesh is filled with ectoderm and endoderm, the latter forming, in sections, a well stained core with a small central lumen, surrounded by tall, somewhat vacuolated cells of the ectoderm which is in direct contact with the surrounding chitin. Where two meshes communicate with each other the ectoderm is seen to be directly continuous. Colcutt¹³ says that in *Hydractinia echinata* the meshes of the deeper part of a colony are filled with degenerating coenosarcal masses; but in the present species the deepest meshes are filled with coenosarc which is in no way different from that of the upper part. It has already been mentioned that the meshes of the deepest layer are closed by a thin, deeply staining chitinous membrane,

¹¹ *Ibid.*: '09, p. 22.

¹² Goto: '97

¹³ Colcutt: '98, p. 85.



HYDRACTINIA SODALIS

Fig. 1 A colony without polyps, with the enclosed gastropod shell (*Columbella* sp.) on one side. Nat. size.

Fig. 2 A colony without polyps on a gastropod shell of one of the *Buccinidae*. Nat. size.

Fig. 3 Transverse section of the chitinous skeleton at right angles to the mouth of the shell. 60 diam. The upper side is the outer.

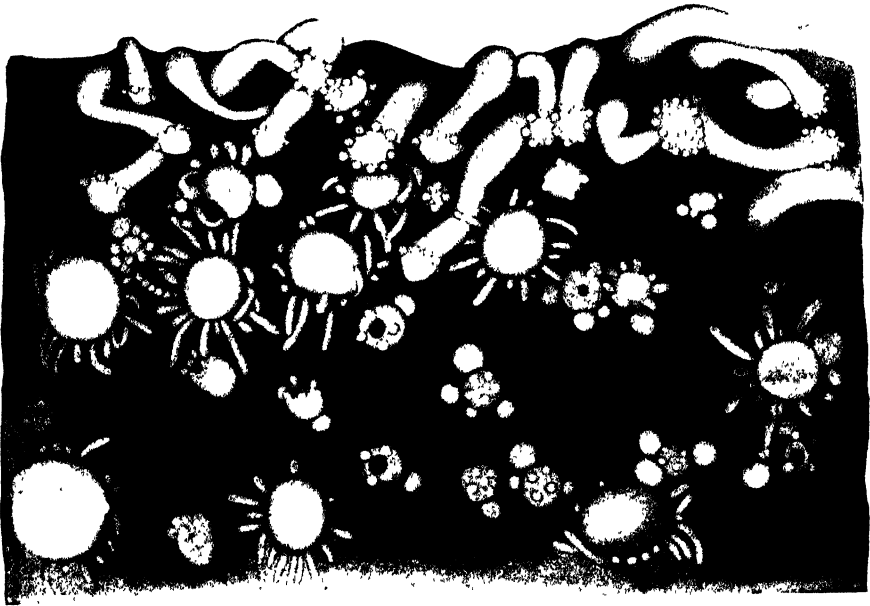
Fig. 4 Longitudinal section of spine, axial part. 60 diam.

so that the coenosarc does not come in direct contact with the body of the hermit crab. It goes without saying that the endoderm of the whole colony is continuous throughout. The outer surface of the colony is entirely covered over by an ectodermal layer, which is comparatively thick in the superficial furrows already mentioned and very thin on the ridges, so that the latter appear as if directly exposed. In this superficial layer of ectoderm are seen numerous endodermal tubes, from which the different forms of polyps take their rise. The entire colony may therefore be regarded as consisting of two sets of sponge-works, the chitinous and the coenosarc.

The colonies are, so far as I have observed, unisexual; but hermaphrodite gonophores, containing both ova and spermatozoa, are occasionally found. A similar case is mentioned by Bunting in *Hydractinia echinata*.¹⁴

The individuals of a colony are composed of the following forms: (a) gastrozooids, (b) dactylozooids, (c) blastostyles, (figs. 5 and 6). The gastrozooids again occur in three apparently different forms. Two of these were noticed by Inaba and regarded by him as gastrozooids and blastostyles respectively; but a comparison of a large number of these polyps has convinced me that they are simply different conditions of one and the same form. By far the larger number of polyps are long and slender, the tentacles very numerous and arranged in several whorls, the hypostome very prominent, hemi-ellipsoidal in form and with a small mouth in the center (fig. 7). In sections the endoderm of the tentacles is seen to be separated from that of the gastric cavity by septa of supporting lamella. These gastrozooids are very numerous and are found all over the colony, on the general surface as well as on the spines (fig. 5). In most of the specimens they are especially abundant on the large spines on the lower surface of the colony i.e., that side on which the colony would slide along when the hermit crab moves on. Sometimes they form such thick tufts on the spines that the latter are well nigh hidden from view. The

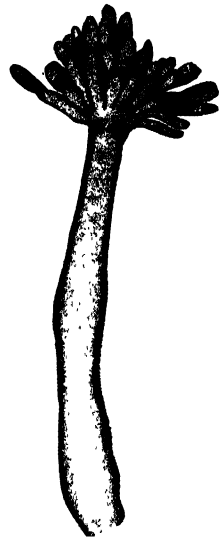
¹⁴ *Loc. cit.*: '94, pl. 9, fig. 7



6



6



7

HYDRACTINIA SODALIS

Fig. 5 A colony with polyps. Nat. size.

Fig. 6 Surface view of a portion of a colony adjoining the mouth of shell; spiral zooids, young nutritive zooids and blastostyles. 30 diam.

Fig. 7 Nutritive polyp. 30 diam.

second form of gasterozooids is also found anywhere on the colony without any regular arrangement, but generally they are far less numerous than the first form. They are thick and comparatively short, the hypostome is large and prominent, the mouth is widely open, and the tentacles are arranged strictly in a single whorl and usually less contracted than in the first form (fig. 8). At a glance these appear to be essentially different from the first form and were so regarded by Inaba, the different arrangement of the tentacles being especially noticeable; but close observations have shown that they are connected by all degrees of intermediate stages with the first form. In fact the polyps of the second form are those that have gorged themselves with prey. It is true that in none of my sections of these polyps have I been able to recognize the nature of the prey, but the gastric cavity was filled with a half digested material. It is rather hard to understand how the different arrangements of the tentacles pass so smoothly into each other, but when we take into account the great elasticity of the body wall such a feat is not impossible. The third group of gasterozooids includes young ones without a mouth and with a single whorl of tentacles. These are especially numerous near the mouth of the chitinous shell (fig. 6).

The dactylozooids are long and slender, and, when numerous, form a thick zone along the mouth of the hydroid shell (fig. 5); their number appears to vary a great deal in the different colonies, only a few being found here and there in some. They are usually situated close to the margin of the chitinous shell but are sometimes found also on the inner surface at a short distance from the mouth. The individual polyps are subject to some variation. Stechow¹⁵ describes them as having a mouth, and with nearly the whole hypostome bearing short tentacles. I find that the form and number of these tentacles are subject to great variation. Thus, in the portion of a colony reproduced in fig. 6, most of the spiral zooids are seen to be provided with only a single row of tentacles which are so exceedingly short as to appear like so many simple knobs, but one of them has many tentacles which are

¹⁵ *Loc. cit.*: '09, p. 22

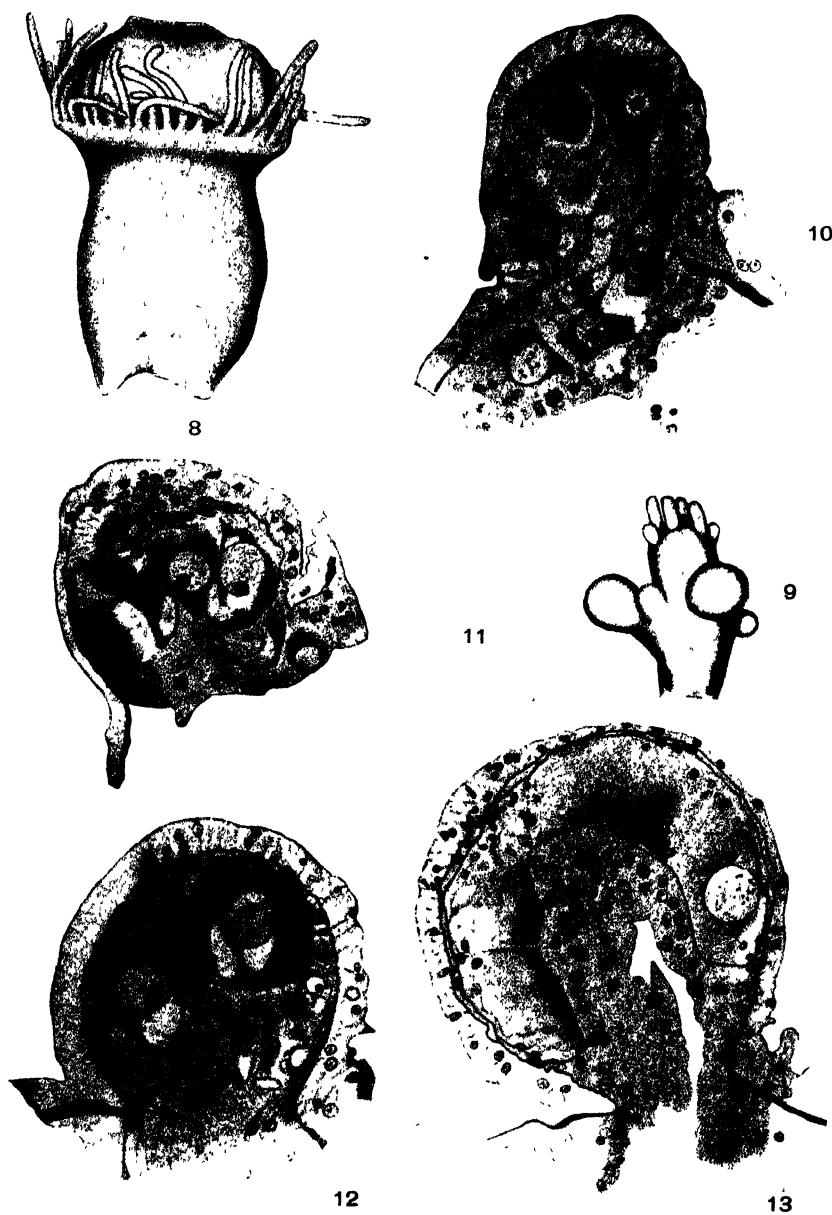
arranged in several rows and cover nearly the entire surface of the hypostome. Now, in some other places and colonies spiral zoöids, of the latter form are much more numerous, and the tentacles are frequently longer. Again, there are sometimes found near these spiral zoöids true gasterozoöids which are much more slender than their fellows and with shorter tentacles, forming in fact a transition to the true spiral zoöids. The latter have the gastric cavity well developed, but in no case have I been able to find the mouth, although the above observations would not justify one in entirely denying its presence, the more so as there are all degrees of transitional forms.

The blastostyles are found in large numbers on many of my specimens, fig. 9, which were all collected in the first part of the month of April, both on the general surface of the chitinous shell and on the spines. They appear, however, to be absent from the apical part of the spines. They are considerably smaller than the other polyps (figs. 6, 9), fusiform in shape, with a small but variable number of short tentacles without any definite arrangement, and with an inconspicuous hypostome destitute of a mouth. They are, as already mentioned, generally unisexual, but occasionally hermaphrodite ones occur. For instance, among 51 blastostyles taken at random from three colonies, viz., 13 from colony *a* (all ♀), 20 from colony *b* (mostly ♀) and 18 from colony *c* (all ♂), there was only one hermaphrodite gonophore (from colony *b*). The gonophores appear to arise from a somewhat narrowly defined zone about midway along the length of the blastostyle (fig. 9). The female gonophores are spherical, the male ellipsoidal, but the elongation in the latter is so slight that the difference is noticeable only in sections passing through the long axis of the gonophore. When there are many gonophores on a blastostyle they are arranged roughly in a circle, although some of them may be superposed. In the following will be given an account of the development of the gonophores as made out from a complete series of stages. My results differ from those of previous observers working on *Hydractinia echinata*.

Female gonophores

I have never been able to find ova outside the blastostyles, but in the neighborhood of very young gonophore buds which are simple outpocketings of the body-wall they are fairly numerous in the endoderm. I have not been able to make out satisfactorily whether they are formed by division of a single endoderm cells or by their bodily transformation, but my observations so far incline me to the latter alternative. On this point Smallwood¹⁶ has come to the same conclusion in *H. echinata*. Fig. 10 represents a young female gonophore bud, a simple protuberance of the body-wall of the blastostyle, its cavity filled up by endoderm cell which do not show any distinct epithelial arrangement, and containing several ova in the spacious interstitial cavities. Several ova are also found in the endoderm of the blastostyle in the immediate neighborhood of the bud. Generally speaking, the ova in the latter position are younger than those in the bud, although exceptions are not rare, of which one instance is seen in fig. 10. The young ova are very conspicuous in sections by their size, the deeply staining granular protoplasm and large vesicular nucleus containing a nucleolus and a diffuse, faintly staining chromatin network. They are generally irregular in shape, but whether this is to be ascribed to an active movement on their part or is due to a mere passive adaptation to the shape of the spaces in which they lie cannot be determined. It is at least not necessary as Goette ('07) maintains, to assume an active amœboid movement to explain the passing of the young ova into the gonophore buds. At a slightly later stage (fig. 11) in which the bud is solid, is seen the beginning of the bell nucleus, or as Goette¹⁷ prefers to call it "Innendetoderm," or "Parectoderm." This is formed by the proliferation of the cells at or near the apex of the bud, in consequence of which the supporting lamella is pushed inward. Thus a fold of the latter is formed, which is continuous all around the bud, the bell nucleus soon becoming more or less cap-shaped and an endodermal lamella forming. Previous observers¹⁸ have either not mentioned or denied

¹⁶ Smallwood: '09, p. 210. ¹⁷ Goette: '07, p. 78.



HYDRACTINIA SODALIS

Fig. 8 Nutritive polyp. 30 diam. Fig. 9 Blastostyle. 45 diam.
Figs. 10-13 Developmental stages of the female gonophores. 370 diam.

the existence of the endoderm lamella in *Hydractinia echinata*, but there cannot be the least doubt of its presence in the species we are considering¹⁹ On a close examination of the figures reproduced by Bunting²⁰ there is hardly any doubt that the parts marked *bn* in figs. 6, 10, 11, 12, 13 are endoderm lamellæ, although, strangely enough, nothing is said about them either in the text or in the explanation of figures. The lettering would suggest that she probably regarded them as parts of the bell-nucleus. Bunting and Goette must have missed the important stages. It must be remarked, however, that the endodermal lamellæ are never more than one cell layer thick and no trace of canals is ever developed. In the stage represented in fig. 11 the ova still lie entirely in the endoderm, although some of them are in close contact with the supporting lamella. In a somewhat later stage, represented in fig. 12, the endodermal lamella is very distinct; nearly all the egg cells have passed out of the endoderm into the inner ectoderm and one is seen to be just passing through the supporting lamella, causing a big temporary hole in it. With the passing out of the egg cells from the endoderm of the bud a cavity becomes apparent in the latter, the endoderm cells assume an epithelial arrangement, and the lumen of the bud becomes continuous with the gastric cavity of the blastostyle. The cells of the inner ectoderm have become scattered between the egg cells and between these and the supporting lamella; but they are as yet comparatively few. Another point that deserves notice in this stage is the eccentric position of the opening by which the outer ectoderm communicates with the inner. This condition occurs frequently, although it is not invariable, and the same can be found in many gonophore buds of a much younger age. At the stage represented in fig. 13 the gonophore has grown much larger, the endodermal lamella has become thinner, the spadix

¹⁸ Bunting: '94, Colcutt: '98, Goette: '07.

¹⁹ It appears to me that Agassiz ('60 and '62, pl. 16) shows in his fig. 8 the presence of the endoderm lamella, marking it *b*¹ and calling it the "inner wall of the medusa." The figs. of Allman ('71, p. 32, and pl. 15, fig. 6) are not clear enough to enable one to say anything definite on this point.

²⁰ Bunting: '94, pl. 18.

has become distinctly elevated; the opening by which the inner ectoderm communicates with the outer is also eccentric in position but has become smaller, and shows signs of being closed up, as may be inferred from the presence of a deeply staining substance similar to that of the supporting lamella which spans the space lying between the edges of the endodermal lamella. The cells of the inner ectoderm are not very numerous, and their nuclei can be clearly distinguished between the egg cells. The young ova that are sometimes found in the endoderm of the blastostyle close to the base of the gonophore bud at this stage are probably reserved for the next bud. In the final stage here reproduced (fig. 14) in which some of the ova are nearly ripe, the gonophore is nearly spherical, the spadix is very conspicuous and may sometimes show a tendency to branch and grow outward between the egg cells. The endodermal lamella has become exceedingly thin and the nuclei contained in it are recognizable with extreme difficulty, so that at such an advanced stage the presence of an endodermal lamella can hardly be made out. The failure on the part of previous observers to recognize the existence of the endodermal lamella in *Hydractinia echinata* is probably due to the fact that it becomes exceedingly thin at a very early stage. The opening at or near the top of the gonophore by which the inner and the outer ectoderm communicate with each other is probably never closed entirely; for in a nearly ripe female gonophore examined to settle this point, there was found an opening at the place in question which ran through two sections (each = $7.5\ \mu$). The opening occupied the centre of a septum consisting entirely of the supporting lamella, the first formation of which was seen in a previous stage (fig. 13). Another fact that must be noted in this stage is the great increase in number of the cells of the inner ectoderm and their different appearance. So far these cells had a naked protoplasm, but now most of them have developed a distinct cell wall, and by mutual pressure have assumed a polyhedral form. The few cells which are still seen in fig. 14 to be destitute of a membrane probably develop it later. Frequently the egg cells are separated from one another by wide spaces completely filled with these cells, which thus almost deserve the name

of "follicle cells" (Nährzellen of Weismann '83). In the gonophore represented in fig. 14, one of the ova has a very distinct layer of ectosarc, hence it was probably ripe. I have never observed a cell layer on the inner side of the endodermal lamella, although such is suggested in certain stages of the male gonophore (vide infra).

Male gonophores

As already stated, the male gonophores are generally found in a different colony from that of the female, although hermaphrodite gonophores are occasionally found. The position and mode of origin of the male gonophores are in no essential way different from those of the female. In fig. 15 is reproduced a very young bud. It contains a lumen which is a direct continuation of the gastric cavity of the blastostyle, and numerous mitotic divisions are taking place at the apex both in the ectoderm and endoderm, but especially in the latter. The cell proliferations in the ectoderm give rise to the rudiment of the inner ectoderm, which is seen to be a darkly stained mass of cells with granular protoplasm and without any distinct cell boundaries. In the section here reproduced it has just slightly pushed the supporting lamella inward. The divisions in the endoderm give rise to the germ cells. These are mostly formed near the apex of the bud, but some appear to migrate into it ready formed from the adjoining endoderm. One such case is shown in fig. 15 (*gm.*) In the next stage here reproduced (fig. 16) the inner ectoderm has been formed and is seen to form a cap-shaped mass of cells communicating with the outer ectoderm by a comparatively small opening, which is bounded on all sides by the apical margin of the endodermal lamella. The position of this opening is mostly apical but it may be shifted considerably to one side, as in the female gonophores. The cell proliferation in the endoderm has become so intense as to greatly obscure the internal cavity, although this appears to be always present more or less. In my opinion, the germ cells are still all in the endoderm in the section reproduced in fig. 16, so that all the nuclei contained in the cap-shaped mass above mentioned



HYDRACTINIA SODALIS

Fig. 14 A nearly ripe female gonophore. 230 diam.

Figs. 15-17 Developmental stages of the male gonophore. 370 diam.

Fig. 18 A nearly ripe male gonophore. 230 diam.

are truly ectodermal in nature. The germ cells are very numerous in the endoderm, although it is hard at this stage to distinguish them individually from cells that remain definitively endodermal. They are however characterized by large vesicular nuclei. The gonophore grows larger, the inner ectoderm spreads out more and more, the germ cells passing through the supporting lamella come to lie in the inner ectoderm, and the endoderm rises up in the center of the bud to form the spadix, until the stage represented in fig. 17 is reached. A very conspicuous feature of this stage is the inner ectoderm. This forms a conspicuous deeply staining mass in and around the original opening by which it communicated with the outer ectoderm. In the section here reproduced the opening has been largely filled by a membranous septum of supporting lamella. Numerous small nuclei are scattered between the germ cells, staining more deeply and diffusely than the latter, and doubtless belong to the cells of the inner ectoderm. A similar layer of the inner ectoderm capping the top of the germ cells is described and figured by Goette in *Hydractinia echinata*²¹. The endodermal lamella has become much compressed and dense, but the nuclei can still be distinctly seen. In this as in the previously figured stage the outer ectoderm is greatly thickened at the top of the gonophore; this becomes less conspicuous in later stages and may entirely disappear at last. In fig. 18 is reproduced a male gonophore which is nearly ripe, and there is no difficulty in connecting it with the previously described stage; for the same parts are present in both, though much altered. The spadix has become greatly elongated and irregularly club-shaped, the tall endodermal cells presenting a typical epithelial arrangement, and enclosing a central cavity directly continuous with that of the blastostyle. The endodermal lamella is exceedingly thin, but can be clearly distinguished as a distinct layer separating the two supporting lamellæ enclosing it and directly continuous with the endoderm of the spadix at the base of the gonophore; the nuclei can be observed with extreme difficulty if they are present at all. The opening between the inner and the outer ectoderm was present in a

²¹ Goette: '07, p. 73, pl. 6, fig. 132.

single section ($=7.5 \mu$) in a nearly ripe gonophore examined for the purpose, the original communication being mostly cut off by the formation of a membranous septum of supporting lamella. It may also be remarked that the position of this opening may be apical or eccentric both in young and mature gonophores. The larger part of the contents of the gonophore at this stage consists of the germ cells which have become very small by repeated divisions. Numerous karyokinetic figures are however present, showing that the process of division is still actively going on. The figures probably belong to maturation stages. Careful observation shows the presence of the nuclei of the inner ectoderm cells here and there between the sperm cells, distinguishable by their smaller size, more or less elliptical shape and especially by their deeper and diffuse staining. The ripe male gonophores are, as already mentioned, slightly ellipsoidal in shape.

Relationships with other forms

In regard to the soft parts nothing particular need be said on the relationships of the present species, but the skeleton appears to lead us to a better understanding of some of the forms previously described, including certain fossil ones. Of the species having skeletons of the same general structure as *H. sodalis*, Stechow²² mentions *Hydractinia calcarea* Carter,²³ *Hydractinia angusta* Hartlaub ('04), *Hydractinia dendritica* Hickson & Gravely,²⁴ *Hydrodendrium gorgonoide* Nutting²⁵ and *Clathrozoön wilsoni* Spencer.²⁶ Again Nutting²⁷ mentions the following families as being more or less closely related: Hydractinidæ, Ceratellidæ (Solanderidæ), Hydrodendridæ, Tubidendridæ (a new family to be described), Hydroceratinidæ and Milleporidæ. It seems to me that of the described species of *Hydractinia*, *H. arborescens* Carter is most nearly related to *H. sodalis*, if indeed it is not identical with the latter. Carter²⁸ had a single specimen of this species which "now

²² *Loc. cit.*: '09, p. 22.

²⁴ Hickson & Gravely: '07, p. 9.

²⁶ Spencer: '90, p. 123.

²³ Carter: '77, p. 50.

²⁵ Nutting: '06, p. 936.

²⁷ *Loc. cit.*: '06, p. 938.

belongs to the British Museum, and was found, without any label or indication of its locality, among the late Dr. Bowerbank's collections." Carter ('78) however gives the locality as Polynesia with a query, and Steinmann²⁹ gives it as "wahrscheinlich die Philippinen." The figures both of Carter and Steinmann clearly show its close resemblance in the general form of the chitinous skeleton to *Hydractinia sodalis*, and it is not going too far to assume that the internal structure must also be closely similar, especially as Carter's description of the skeleton of *Hydractinia echinata*³⁰ shows essentially the same arrangement of parts, as in *Hydractinia sodalis*. Here it may be mentioned that Carter's description of '73 is much more accurate than that of '77, as may be seen by comparing the two descriptions with the results obtained by Colcutt ('98) through the use of modern methods. The later paper of Carter ('77) appears to me to have been influenced too much by a desire to bring out the supposed affinity with certain fossil forms (other than *Hydractinia*), which must in my opinion be said to be at most only remote. Renewed examination of the various fossil forms mentioned by Carter and Steinmann, especially if microscopical sections are prepared, will probably throw light on this question. In Carter's specimen of *H. arborescens* the hydroid skeleton did not entirely cover the shell which was that of *Fusus sulcatus*,³¹ but this is clearly a point of secondary importance. Again in *Hydractinia levispina*³² is clearly seen, as pointed out by Steinmann,³³ the tendency of the hydroid skeleton to grow beyond the mouth of the gastropod shell to form a "shell" of its own, and the presence of the same tendency in *Hydractinia echinata* can be inferred from Carter's description.³⁴ If now we compare the chitinous skeleton of *Hydractinia* with those of *Solanderia*, *Clathrozoön* and *Hydrodendrium*, they are all found to be on essentially the same plan derived from the well known fundamental structure of the hydroid colony. Are we to infer from this that they are all closely related phyletically? There can be no

²⁹ *Loc. cit.*: '78, p. 299. ³⁰ Steinmann: '78, p. 109. ³¹ Carter: '73, p. 2.

³² I.e., according to Steinmann. Carter gives it as *Phos senticosus* or *Fusus sulcatus*.

³³ Carter: '73, pl. 1, fig. 1.

³⁴ *Loc. cit.*: '78, p. 109.

doubt that the corresponding parts are homologous in a general way, but it would be in my opinion a mistake to look upon these forms with complex skeleton as more nearly related to each other than to those without it. To clarify this point a much more detailed knowledge of the structure and development of these forms than we possess at present appears to me necessary. So far as I can see at present the development of a complex skeleton in these forms appears to have gone on independently in the different species; in other words, the resemblance is one of homoplasy. As to the relationships of the fossil forms mentioned by Carter³⁵ and Steinmann ('73), I have nothing to say, because I have not been able to study them. So far as I can see from the descriptions, however, their affinities to the living hydroids appear to me to be more remote than is supposed by Carter and Steinmann, with the exception of the species of *Hydractinia*.

2. *HYDRACTINIA SPIRALIS*, N. SP.

I can deal very briefly with this species, because the gonophores appear to be in all essential respects exactly like those of the preceding species. The form was first described by Inaba³⁶ as a species of *Podocoryne* as follows:

3. *Podocoryne* sp. (figs. 5, 6, 7).

Trophosome.—Hydrorhiza consisting of numerous parallel small tubes, the perisarc covering them being fused together and forming a strong lamella. From this lamella spring small pointed chitinous spines. Hydranths also springing in large numbers from the lamella; they are of two forms, those without reproductive organs large and with 12-18 tentacles; those bearing reproductive organs small and with 4-8 tentacles.

Gonosome.—Medusoid, growing from the hypo-tentacular part of the hydranth; imperfectly developed and incapable of swimming, merely with four radial canals and a circular; manubrium, however, full of genital cells, enlarged and entirely filling the cavity of the umbrella.

Color.—Hydranth colorless; perisarc reddish brown.

³⁵ *Loc. cit.*: '73, p. 2.

³⁶ *Loc. cit.*: '77, and '78.

³⁶ Inaba: '09, p. 98.

Locality.—Between Misaki and Jogashima, 3 *hiro*³⁷ deep, covering worm-tubes resembling gastropod shells, inhabited by a hermit crab in place of the dead worm.

Date.—April, 1889. Collected by Mr. Shishido.

The hydrorhiza is very strongly built but very thin, hence it is easy to prepare sections of it. The original worm tube was probably very delicate and hardly leaves any of its traces; the whole lamella consisting of the creeping hydrorhiza of the Podocoryne. Perisarc not elevated in the form of a bowl at the base of the hydranths. The chitinous spines are hollow and filled with a coenosarc consisting of the two layers and ending blindly. Some of the spines have incomplete or exceedingly thin apices; these are probably growing ones.

The upper surface of the hydrorhizal lamella is covered by a naked coenosarc, which has been described as consisting only of the ectoderm, but which, according to my own observation, appears to contain also the endoderm; this point requires reëxamination.

The hydranths are very small, the larger ones being 1.5 mm. high and the smaller ones bearing medusoids not being over 0.5 mm.; the spines are 0.5–0.7 mm. high.

Of the medusoid gonophores there are generally two on a hydranth, the lower one being the larger; the full grown medusoid comparatively large, with a diameter of over 0.2 mm.

This is a very interesting species and probably new. The genus Podocoryne usually produces free medusae and Hydractinia gonophores which are never detached. The present species is intermediate on this point, the reproductive organs being medusoid and never detached. Hence it may be somewhat doubtful to which of these two genera to refer it, but the presence of the four radial canals in the reproductive organs (fig. 7), and of tentacles on the hydranths bearing them, or blastostyles, leaves no doubt that it belongs to the genus Podocoryne.

According to Allman, there is no doubt that the *Hydra aculeata* of Wagner is a Podocoryne. In this species the medusa does not complete its development, being never free, although there are four radial canals and four tentacles on the edge of the umbrella; hence it is evident that there are several stages in the development of the medusa within the genus Podocoryne. The description of *P. aculeata* agrees fairly well with my specimens; possibly they are the same species. R. Wagner is stated to have obtained it in 1833 on the coast of the Adriatic Sea, but it has not been obtained since.

³⁷ A "hiro" is an arm-span, equal to about 1.6 m.

I shall confine myself to the points on which my own observations differ from those of Inaba, but in criticizing his descriptions it must be borne in mind that his work was done under some peculiarly difficult conditions and with insufficient literature at his command.

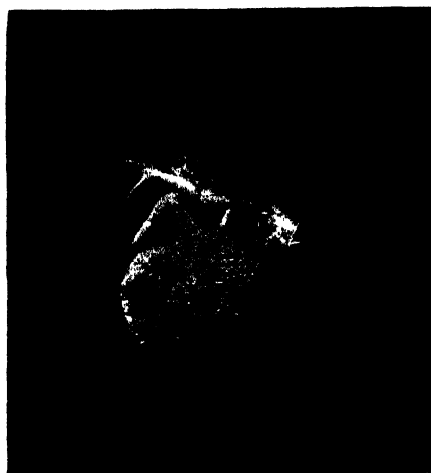


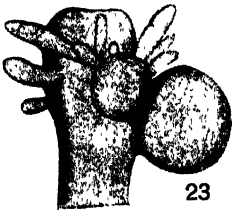
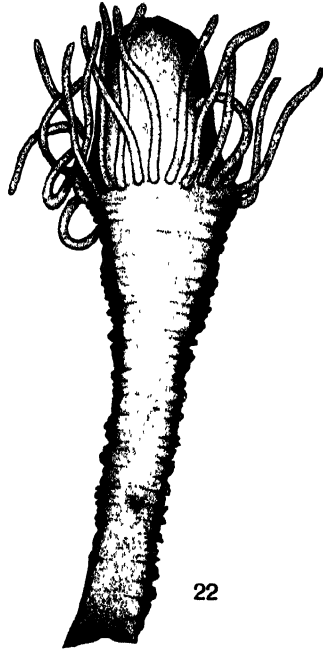
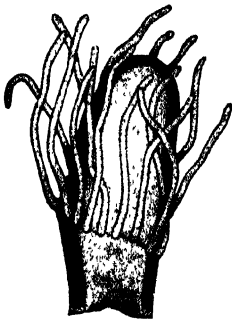
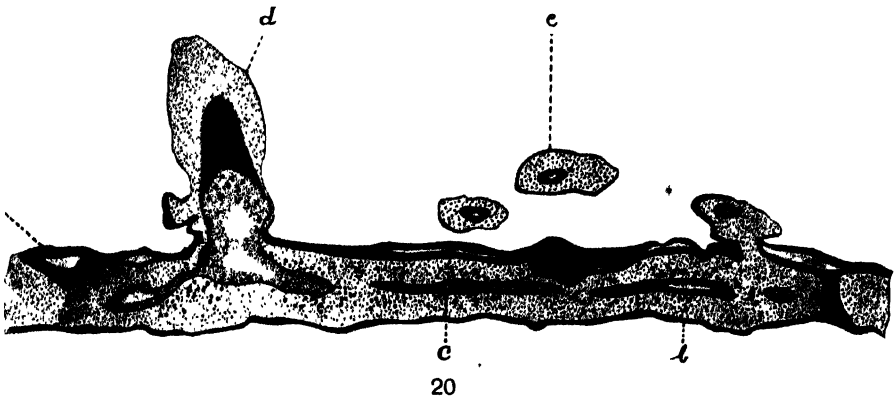
Fig. 19 A colony with polyps. Nat. size.

In the first place, what Inaba thought was a worm-tube is really the chitinous skeleton of the hydroid. It has the shape of a gastropod shell, is much more regular, judging from my specimens, than the corresponding structure in *H. sodalis* (fig. 19), and by far the larger part of it is soft and of a dirty greenish hue. The inner surface is perfectly smooth, and when examined with a hand lens shows exceedingly fine reticulations, due to the inner structure to be mentioned later. The thickness of the hydroid shell varies a good deal in different parts, being very thin near the mouth but becoming as thick as 2 mm. or more near the apex. On the outer surface are found much dirt, minute sand grains, diatoms, etc., and much of the thickness of the subapical part of the shell just mentioned appears to be due to the presence of these extraneous bodies. The chitin of the skeleton is flexible but very strong, so that it is relatively more difficult to obtain good serial sections

of this species than in *H. sodalis*. These show that in the thinner part of the shell its hard parts consist of two parallel chitinous lamellæ about 7-22 μ in thickness and 0.1 mm. apart, connected together at many points by strong chitinous trabeculæ of varying thickness (fig. 20). It was probably these trabeculæ that Inaba mistook for surface spines. The inner surface of the shell is bounded by the inner of the two lamellæ just mentioned; the outer surface is also largely bounded by the outer of the two lamellæ, but in many places chitinous tubes of the structure generally seen in monosiphonic hydroids take rise from the outer lamella and creep about on the outer surface of the shell in close apposition to the former. The interspaces between the two chitinous lamellæ are completely filled with ectoderm which contains numerous ramifying endodermal tubes forming a network, to which is due the appearance of the inner surface of the shell mentioned above. The superficial tubes above referred to are filled with the continuations of the endoderm and ectoderm filling the interlamellar cavities, exactly as in monosiphonic hydroids; but I have never seen them give rise to polyps, although my observations on this point are confessedly incomplete. As to the thicker part of the shell, I have not been able to obtain satisfactory serial sections owing to the presence of much dirt and sand grains. Where the chitin is of some thickness in any part of the shell it is seen to be composed of several superposed layers. According to my observations there is no coenosarc on the surface of the shell, and the relations of the soft part and the chitin are in this species nearer those found in the typical hydroids than in *H. sodalis*.

In this as in the preceding species there is always a small gastropod shell in the apex of the hydroid skeleton; in one case it was that of a species of *Fusidæ* only 6.5 mm. long, although the hydroid shell was 25 mm. in length. It is interesting to note that this species never grows so large as the preceding, although found in the same localities. The mother whorl of the hydroid shell is relatively very large and the turret very small.

So far as I have observed, the polyps are of two kinds, nutritive and reproductive. The former, or gasterozooids, may be found in any part of the colony, but most frequently they are especially



HYDRACTINIA SPIRALIS

Fig. 20 Transverse section of a colony at right angles to the mouth of the shell. The details of the cellular parts are not shown. *a* outer chitinous lamella, *b* inner chitinous lamella, *c* endodermal tube, *d* blastostyle, *e* superficial hydrorhiza. 90 diam.

Fig. 21 Nutritive polyp. 45 diam.

Fig. 22 Nutritive polyp. 45 diam.

Fig. 23 Blastostyle. 45 diam.

numerous on the columnellar side of the last whorl, i. e., the side on which the hermit crab would slide along in locomotion. On the thinner part of the shell they are always very short and comparatively thick, with a single row of filiform tentacles 20-25 in number. The hypostome is very prominent and as long as the body when the mouth is closed, but flattened out when the latter is wide open (fig. 21). On the thicker part of the shell there are gasterozooids of a quite different shape, with a slender funnel-shaped body, a prominent hypostome and a circle of about 30 tentacles (fig. 22). The relative numbers of these two forms of gasterozooids appear to vary a great deal according to colonies, there being no essential difference between them.

The blastostyles are found where the gasterozooids are few, so that we may broadly speak of blastostyle areas and gasterozooid areas, although the two are not rigidly separate and pass into each other without any demarcation. The older blastostyles are difficult to recognize owing to the great development of the gonophores which they bear, and by which they are more or less pushed aside from their natural position. In young blastostyles bearing a few young gonophores their form stands out very clearly, and it is then seen that they are but little different from the slender gasterozooids above mentioned, except that they are smaller all around and the tentacles are less numerous, there being only 6-12 on each (fig. 23). The hypostome is relatively very large, and the mouth is present in older ones. The gonophores are borne about midway between the base and the tentacles, and on an older blastostyle there may be as many as twelve or more of them. So far as I have observed the gonophores develop essentially in the same way as in *H. sodalis*. There are no canals, radial or circular, at any time and although in some gonophores the ectoderm is much thickened at the top and contains numerous nettle cells, there are no tentacles.

This species is common in different parts of the Bay of Tokyo and in the vicinity of Misaki, although it appears to be less so than *H. sodalis*.

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THE DEVELOPMENT OF AN APODOUS HOLOTHURIAN (CHIRIDOTA ROTIFERA)

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SIX FIGURES

In the summer of 1897, while enjoying the privileges of the Johns Hopkins Biological Laboratory at Port Antonio, Jamaica, I collected a number of specimens of a small holothurian (*Chiridota rotifera*) on the reef at Titchfield Point, where it lived in the sand beneath the broken pieces of coral rock. This species is of more than usual interest because like its near relative, *Synaptula hydriformis* [= *Synapta vivipara* (Oerst.)], the eggs undergo their development in the body-cavity of the mother and the young are born at an advanced stage. Among the specimens which I collected, were half a dozen, which contained young in the body-cavity, and it was my hope that I might be able to study the development of *Chiridota* in the same way and to the same extent as I had already done with *Synaptula*. The material collected in 1897 was not sufficient, however, to make such a study possible, and it was accordingly put aside until another opportunity should present itself for collecting *Chiridota*. In April, 1899, I made a brief visit to Bermuda and there I found the desired holothurian quite common. Not having facilities for laboratory work, I simply gathered all the adult *Chiridotas* I could find and preserved them in alcohol. In November, 1902, and again in March, 1909, it was my good fortune to be able to revisit Jamaica, but on neither occasion did I find *Chiridota* at all common, and only a few small specimens were obtained.

Dr. Brooks was greatly interested in the discovery of *Chiridota* at Port Antonio in 1897, and desired me to complete my study of

the development if possible. In later years, he expressed the hope that I would publish such results as I had obtained. When, therefore, it was suggested that his former students publish a volume of zoölogical papers as a tribute to his memory, it seemed to me especially appropriate that I should prepare an account of the development of Chiridota, so far as the material at hand would permit. Fortunately the Bermudan material has proved to contain some much-desired stages, in an excellent state of preservation, and has thus supplemented that from Jamaica quite satisfactorily. The recent papers by Östergren ('07), Becher ('07, '08, '10) and Edwards ('09) have been a further stimulus to my work, arousing, as they have, renewed interest in the phylogeny of holothurians. The present paper consists of two parts, one devoted to the account of Chiridota rotifera and its development, the other to the bearing of the facts there set forth, on the phylogeny of the class.

PART I THE NATURAL HISTORY AND DEVELOPMENT OF CHIRIDOTA ROTIFERA

Few species of Chiridota are better characterized than *rotifera*, the only member of the genus known to occur in the West Indian region. It was first described by Pourtales in 1851 from Florida specimens and is easily recognized by its small size, distinctive color, numerous wheel-papillae, and the minute rods in the skin. Although large individuals when fully extended are nearly 100 mm. long, the great majority of specimens are less than 60, even when living; preserved specimens are usually 30-50 mm. in length. The color in life is pale flesh-red, with either a pink or a yellow tinge, but this ground color is obscured by the convex, white spots, known as "wheel-papillae," formed by the clusters of calcareous wheels in the skin. These wheel-papillae are numerous, often crowded, and occur all over the body; in young individuals, however, they may be few and scattered on the ventral side. The twelve tentacles are white or cream-colored and have 8-14 digits. In alcoholic specimens the colors are usually deepened and are fairly persistent, but after some months they

are apt to fade and the specimens may become entirely bleached. The calcareous deposits in the skin are of two kinds; the six-spoked wheels, usually about .12 mm. in diameter, characteristic of the genus, which are collected in the wheel-papillae, referred to above; and small, somewhat flattened, curved rods with enlarged and often slightly branched ends, which are about .05 mm. long and are scattered all through the interambulacra.

The habitat of this little holothurian, so far as my experience goes, is always coral sand, in shallow water, just inside a reef exposed to surf. Here, under a slab of rock or some similar shelter, it finds a congenial home and often (especially in Bermuda) a number of specimens will be found occupying the limited area beneath a single rock. In Jamaica, the companions of *Chiridota*, in such a spot, are the two little echini, *Brissus unicolor* and *Echinoneus semilunaris*, and occasionally a small synaptid; but in Bermuda, the usual companion is *Leptosynapta roseola*. *Chiridota* is not hardy, and specimens brought into the laboratory at Port Antonio lived but a few hours and were never very active. Curiously enough the pentactula larvae were much more hardy than the adults, for they lived more than twenty-four hours after removal from the body of the mother, and showed no effect from solutions of magnesium sulphate which completely narcotized the adults.

Owing to their sensitiveness to changed conditions, observations on the *Chiridotas* in the laboratory yielded no facts of interest. We can only assume that fertilization of the eggs and the birth of the young take place as in *Synaptula*; the study of preserved material has thrown no light on these points. Specimens collected in Bermuda in April, and in Jamaica in July and August, contain young in various stages of development, but whether breeding occurs all the year, as seems to be the case in *Synaptula hydriformis*, is not proven. In every specimen examined which contained young, these were all of approximately the same stage of development, indicating their origin from a single ripening of ova. No evidence of two or more overlapping broods, such as occur in full-grown synaptulas, has been found. The number of young is commonly much greater than in *Synaptula*, for while small *Chiridotas* may contain only a few larvae, the full grown

specimens often contain many score. One Bermudan specimen, less than 50 mm. long (after preservation), contained no less than 522 young, three times as many as I ever found in a *Synaptula*. As no larvae over 3 mm. long were found, and none with more than eight tentacles, it is very possible that the young are born in the eight-tentacled stage, which would be much earlier than birth occurs in *Synaptula*. Further study of living material is necessary to settle some of these interesting points.

The mature eggs of *Chiridota* were not seen, nor were any of the segmentation stages observed; but to judge from the youngest embryos found, the eggs, blastulae and gastrulae, must be very similar in size and appearance to those of *Synaptula hydriformis*. A few specimens of the earliest developmental stage which has been observed were found in a small adult at Port Antonio in 1897 and one of them is shown in fig. 1. It is a uniformly ciliated embryo, within which the hydro-enterocoel has separated from the primitive gut and already shows the constriction by which the hydrocoel is to be formed. This embryo corresponds in all essentials with the similar stage in *Synaptula*.

In the next stage observed, which was also found in a Port Antonio *Chiridota*, the body is a little more elongated and there are indications of bands of cilia longer and more vigorous than those which still cover the embryo. The hydrocoel is well-formed and is connected with the outside by the pore-canal. The enterocoel has already divided and the two parts lie, one on each side of the primitive gut. There is no indication of a mouth. This embryo corresponds closely in all the details of its inner anatomy to the similar stage of *Synaptula* but is noticeably different externally in the absence of a mouth and in the presence of ciliated bands. With the very small amount of material available, I have not been able to determine positively, the arrangement of these bands, but they are very similar to those figured by Semon ('88) for the corresponding auricularia larva of *Labidoplax digitata*.

• The next oldest larva of *Chiridota* was found in one of the Bermudan adults and was not seen alive. It is shown in fig 2. The ciliated bands are quite prominent, especially at the larger ante-

rior end; at the narrowed posterior end, they are less distinct but seem to be confined to the sides and ventral surface; their general arrangement is like that found in the auricularia of *Labidoplax*. The uniform covering of cilia seems to have disappeared with the development of these bands. There is still no mouth but the blastopore remains open. The hydrocoel clearly shows the beginnings of the five primary tentacles and less distinctly the first rudiments of the "secondary outgrowths." On the pore-canal, the thin-walled swelling, regarded as the remains of an "anterior coelom" is very conspicuous. Near the blastopore are several minute, discoidal calcareous bodies. At this stage, the larva of *Chiridota* is rather strikingly intermediate between the corresponding stage of *Labidoplax* (Semon, '88, pl. 6, fig. 3) and that of *Synaptula* (Clark, '98, pl. 11, fig. 15). While the external form is unlike either, the ciliated bands and calcareous particles resemble those of *Labidoplax*, and the general appearance and arrangement of the inner organs correspond to what is seen in *Synaptula*. In the absence of a mouth at this stage, *Chiridota* is quite unique.

A very few larvae, a little beyond the stage just described, were found in a small adult at Port Antonio in 1897. The hydrocoel was beginning to encircle the foregut, the primary tentacles were very conspicuous and the secondary outgrowths were correspondingly large. These larvae are peculiar because, in spite of this development of the hydrocoel, the uniform ciliation of the body still persists and ciliated bands cannot be distinguished.

A large proportion of the larvae found, both at Port Antonio and in Bermuda, were well beyond this stage and have the appearance shown in fig. 3. Four ciliated bands are commonly present, though the most anterior is hard to make out and may be wanting; the other three are not of uniform width or density at all points, but seem to be best developed ventrally. The hydrocoel has completed its growth around the foregut and the primary tentacles are very conspicuous, completely over-shadowing the secondary outgrowths which have developed very little. The polian vessel is very evident in the left ventral interradius. The "anterior coelom" on the pore-canal has reached its maximum development. The right and left coelomic vesicles have coalesced and the result-

ing body-cavity is very distinct. The blastopore has closed and the larval gut has bent forward and then backward again. There is still no mouth, nor did I find any evidence of a differentiated nervous system. The calcareous particles at the posterior end of the larva have undergone marked development. The discoidal plates (fig. 5 *a*) have outgrowths on the margin (5 *b*) which grow out rapidly as projecting rays (5 *c*). The plate is no longer flat but somewhat convex at the center, and the rays, instead of lying in the same plane with it, curve upward and outward towards the surface of the body (5 *d*). The tips of the rays extend laterally (5 *e*) until they finally coalesce into a solid rim, and thus a fully developed wheel is formed (5 *f*). These wheels are about one-twenty-fifth of a millimeter in diameter and have about a dozen spokes, though the number varies from ten to fourteen; one was found with only nine. These wheels are never very numerous and they are never aggregated into papillae but are scattered irregularly in the skin. The remarkable point about them, however, is that while there is no known species of Chiridota in which the adult has wheels with more than six spokes (normally), in the genus *Trochoderma* the wheels have 10-16 spokes and their development is exactly like those of the larval Chiridota. (Compare Théel '77, pl. 2, or Clark '08, pl. 7, figs. 9-12, with fig. 5). Ludwig ('92) has pointed out that the wheels of the Myriotrochiniæ (to which *Trochoderma* belongs) differ from those of the Chiridotinae in having a simple, solid hub. The wheels of the larval Chiridota have the hub simple and solid. In view of these interesting facts, it is quite correct to say that so far as the calcareous deposits are concerned, Chiridota passes through a *Trochoderma* stage. Semon ('88, pl. II, figs. 5 *a-c*) has figured the wheels found in the auricularia, which he considers to be that of *Labidoplax digitata*; they differ only a little from those found in the larval Chiridota rotifera, chiefly in having 16 spokes. It is certainly most remarkable if the larva of one of the Synaptinae really develops wheels as its first calcareous deposits. The occurrence of wheels with many spokes in the larva of Chiridota rotifera has raised the question in my mind whether the larva which Semon supposed to be the auricularia of *Labidoplax digitata* was cor-

rectly identified. These auricularias were not raised from the eggs nor was their development carried beyond the pentactula stage; they were collected in the tow at Naples and no evidence is offered to show that they are really the young of *Labidoplax*. It is true that no *Chiridota* is known from the Mediterranean Sea, but Semon himself discovered at Naples the interesting *Trochodota venusta*, which has wheel-shaped deposits like those of *Chiridota*. I venture to suggest, therefore, that the question as to what auricularia Semon studied is still open, and until further evidence is offered, I must decline to believe that young synaptids ever develop wheel-shaped deposits. This position is strengthened by the fact that in *Synaptula hydriformis* the first calcareous particles to appear are simple, straight rods, from which (except in the tentacles of course) the plates and anchors rapidly develop.

Pentactula larvae of *Chiridota* were found in many adults, both at Port Antonio and in the Bermudan material, and one of them is shown in fig. 4. No young were found between the stage shown in fig. 3 and the fully developed pentactula, but it is not difficult, in the light of what we know about the development of *Synaptula*, to see how the greater complexity has been brought about. The most important changes are at the anterior end, where an invagination has apparently taken place, giving rise to an atrium, in the center of the floor of which the mouth has arisen by further invagination. The growth of the tentacles upward around the mouth leaves the wall of the atrium, for a time, as a conspicuous collar surrounding the five tentacles. The latter are square-tipped and provided with a greatly thickened glandular and sensory epithelium at their free ends, particularly on the outer side. The alimentary canal has pushed backward to the extreme end of the body, where the permanent anus has formed. The "anterior coelom" on the pore-canal is scarcely visible, but the polian vessel, positional organs and radial nerves are all conspicuous. The beginnings of the calcareous ring are also plainly visible. Traces of the ciliated bands may still be seen on the body. Except for these, the atrial collar, the shape of the tip of the tentacles, and the wheel-shaped deposits in the body-wall, the pentactula of *Chiridota* agrees in all particulars with that of *Synaptula*. There

are no traces of radial water vessels and the development of the water-vascular, nervous, sensory, muscular and alimentary systems is, so far as I can see, identical in the two genera. In *Synaptula* the mouth is formed much earlier and the atrium develops on the ventral side instead of at the anterior end, but these differences cannot be regarded as of any particular importance. They are doubtless due to the accelerated development of *Synaptula*.

The living pentactula of *Chiridota* is most interesting to watch, for the tentacles, which serve as sensory, locomotor and feeding organs, are in constant motion, while the surrounding collar seems to be equally active. Possibly its movements may be respiratory, but I am rather inclined to think they are merely the result of the activity of the tentacles. In the older pentactulas, the tentacles are vertically notched at the tip; the depth of this notch increases with the growth of the tentacle, and thus the pair of terminal digits is formed.

None of the *Chiridotas* collected in Jamaica contained young beyond the pentactula stage, but several Bernudan specimens provided older material. The principal changes which occur in the course of growth are the complete disappearance of the ciliated bands and the atrial collar, the marked development of the two terminal digits on the tentacles, and the appearance of groups of the characteristic six-spoked wheels. The calcareous ring is more noticeable and calcareous rods appear in the tentacles. Neither at this stage, nor in the pentactula, are there any "glandular organs" or "contractile rosettes" present in the skin, such as are found in *Synaptula hydriformis* and *Leptosynapta minuta*. At the close of the pentactula stage, the secondary outgrowths of the hydrocoel, which have remained quiescent beneath the radial nerves, begin to show signs of activity. Their subsequent development is exactly as in *Synaptula*, except that they do not grow simultaneously. The first to develop are those under the latero-ventral nerves; these push out dorsal to the nerves and thus give rise at the same time to the sixth and seventh tentacles. This seven-tentacled larva (fig. 6) was first observed by Ludwig ('81) in *Chiridota rotifera*, and subsequently ('98) by the same eminent zoölogist in *Taeniogyrus contortus*. It appears to be a well-

marked stage of development, but the succeeding tentacles are visible before the sixth and seventh are nearly as large as the original five. In the material of *Chiridota* at hand, I have found only three specimens in which the eighth tentacle can be seen. In each of these, the new tentacle is formed by the "secondary outgrowth" beneath the *right* dorsal nerve pushing out dorsal to that nerve. Our eight-tentacled larva, therefore, has two tentacles in each of the dorsal interradii, while the two ventral interradii have only the primary tentacle in each, a noticeably symmetrical arrangement superficially, but one which leaves the left dorsal and mid-ventral secondary outgrowths of the hydrocoel entirely undeveloped. Whether the ninth and tenth tentacles arise, as in *Synaptula*, from the latero-ventral "secondary outgrowths," I have been unable to determine, as no specimens were found with even the rudiments of these two tentacles. Becher ('07) has found an eight-tentacled stage in *Rhabdomolgus*, like that of *Chiridota*, while the adult retains permanently the ten-tentacled stage, like that of *Synaptula*. It is fair to presume, therefore, that *Chiridota* passes through such a stage.

The calcareous particles in the eight-tentacled *Chiridota* larva deserve a word. The rods in the tentacles are fairly numerous and lie parallel to the long axis. The Trochoderma-like wheels are still to be found, scattered chiefly on the posterior part of the body. There are four or five heaps of *Chiridota*-like wheels in the interradii, two or three near each end of the body. While most of these wheels have six spokes, as they should have, some have seven, and a few have eight. It is noticeable that such variations from the normal are much more frequent than in adult *Chiridotas*, where a wheel with more than six spokes is really very rare.

SUMMARY

1. The development of *Chiridota rotifera* is essentially like that already fully described for *Synaptula hydriformis* (= *Synapta vivipara*, Clark, '98).

2. The early larval stages of *Chiridota* (succeeding the gastrula) differ from those of *Synaptula* in the ovoid form, the

presence of ciliated bands and wheel-shaped deposits, and in the absence of a mouth.

3. The pentactula larva of *Chiridota* differs from that of *Synaptula* in the presence of traces of ciliated bands, an atrial collar, wheel-shaped calcareous deposits, and square-tipped or slightly bifid tentacles.

4. The calcareous wheels developed in the larvae of *Chiridota* resemble in their development, in the number of spokes, and in certain details of structure, those which are found in adults of *Trochoderma*, and are essentially different from the true *Chiridota*-wheels, which appear after the pentactula stage. Whether the auricularia larva, with similar *Trochoderma*-like wheels, found at Naples by Semon and considered by him to be the young of *Labidoplax digitata*, really belongs to that species, must be considered still an open question.

5. The development of *Chiridota*, though accelerated by its viviparous habit, is apparently not so rapid as that of *Synaptula*. This is indicated by the later formation and anterior position of the atrium, the much later formation of the mouth, and the more deliberate formation of the second quintet of tentacles.

6. The viviparous habit appears to have been acquired by *Chiridota* much more recently than by *Synaptula*. This is indicated by the much larger number of young in a brood, by their all being of a single age, by their slower development, by their apparently earlier birth, by the retention of the ciliated bands of a free-swimming auricularia stage, by the unequal development of the secondary tentacles, producing seven- and eight-tentacled larval stages, by the more conspicuous and persistent "anterior coelom," and by the absence of larval "glandular organs."

PART II. THE PHYLOGENY OF THE HOLOTHURIANS

In any discussion of the phylogeny of a class of animals, we ought to distinguish so far as possible between the interrelationships of the groups which make up the class, and the relationships of the class as a whole with other classes. Among Echinoderms this can easily be done, for not only is the phylum a remarkably

distinct one, markedly separate from all other animal types, but each of its component classes is equally distinct, truly annectant living forms being practically unknown. The facts from which the phylogeny of holothurians is to be deduced can be set forth more clearly and the hypotheses which their study has led me to adopt can be made more comprehensible if we first consider the interrelationships of the orders of holothurians, and then discuss the relationship of the class to other echinoderms.

The relationship of the orders of Holothurioidea to each other

Östergren, in a recent paper ('07) has set forth quite fully his views on holothurian interrelationships, and as they differ in some important particulars from those held by Ludwig and others, they will serve admirably as a basis for this discussion. He recognizes five orders of holothurians (Dendrochirota, Aspidochirota, Molpadonia, Elaspipoda, Apoda) and I shall, for convenience, here use his names and accept these orders without further discussions other than to say that I am not sure the names are in all cases tenable. Östergren bases his classification on his own extensive morphological and physiological studies (though of course giving due weight to the work of other investigators), and he lays particular emphasis on the functions of organs and their relation to the habits of the animal. He describes his ancestral form (Stammholothurie) as having a soft body-wall strengthened by scattered calcareous plates; creeping about by the contractions of the body musculature; and feeding on the organic matter in mud ("ernährte sich von Schlamm"). It had "twenty (or ten?)" short tentacles, without ampullae, five radial canals and a number of scattered pedicels. The posterior part of the gut (cloaca) served as a respiratory organ, but there were probably no "water-lungs" developed. From such an ancestor, Östergren derives his five classes of holothurians, finding its nearest living representatives among the Elaspipoda; such genera as *Capheira*, for example, differing only a little from this "hypothetische Stammform."

It is not necessary to criticize this theory in detail here, but there are three general criticisms which seem to me to seriously affect

its value. In the first place, Östergren apparently considers simplicity of structure as implying a primitive condition; he scarcely refers (except as regards the absence of feet in Apoda) to the secondary simplicity often produced by changed habits, and which is sometimes called "degeneration;" he certainly has failed to give due weight to the existence of this factor. In the second place, he assumes that the ancestral holothurians were mud-loving and mud-eating forms; if these ancestors were worm-like in habit and structure this view is tenable, but if they were allied to the regular echini, as many zoölogists consider probable, it is hard to maintain; certainly in the Echinoidea, it is only among the highly specialized forms, the spatangoids, that we find mud-loving and mud-eating species. Moreover, there is little doubt that the early Metazoa were all plankton-feeders and many Dendrochirota retain that habit still. It is hard to believe that the class Holothurioidea had not arisen before the competition for food on the floor of the sea led to the use of organic mud as food. In the third place, to find the most primitive and ancestral form of holothurians in the exclusively deep-water group of elasipods is to run counter to one of the principles, which modern oceanographic work has established; namely, that the inhabitants of the abyssal regions are more or less highly specialized forms, the simpler and less modified forms occurring in water of little or moderate depth.

If Östergren's paper errs on the side of overlooking secondary simplicity and of definitely asserting his deductions, no such criticisms can lie against Becher's ('07) exhaustive study of *Rhabdomolgus*. It is difficult to find a theoretical conclusion definitely asserted in Becher's work, and it is almost as hard to find what characters of *Rhabdomolgus*, if any, are to be considered primitive. The evidence for and against the view that a given character is ancestral is carefully set forth and only occasionally is it possible to decide what Becher's own opinion is.¹ The general conclusion

¹ These statements do not apply at all to Becher's later paper ('08) in which his discussion of questions of holothurian phylogeny is very clear and satisfactory. While his conclusions are not wholly in accord with my own it is not necessary to discuss them here.

appears to be that while *Rhabdomolgus* is primitive in some particulars, it is not to be considered as very near the ancestral holothurian. From the point of view of holothurian phylogeny, the most important facts set forth in Becher's paper are the presence of rudiments of radial canals in the adult *Rhabdomolgus*, accompanied by zigzag rows of "tastpapillen," which very possibly represent the remains of pedicels; the interrarial position of the five primary tentacles; and the points of origin of the five secondary tentacles. These facts are all in accord with the conditions which I have found in *Synaptula* and *Chiridota*. They show clearly the close relationship of *Rhabdomolgus* to the other Synaptidae and their common descent from a pedate ancestor with five primary interrarial tentacles. There is also indicated the possibility of an eight-tentacled and asymmetrical ancestral form.

The existence of ancestral asymmetry is questioned by Edwards ('09) who suggests that, since he found in *Holothuria floridana* a different origin for tentacles 1-5, from that found by Ludwig ('91) in *Cucumaria planici*, it will not do to assume an identical development for all pedate holothurians even in the early stages. Edward's discovery that the five primary tentacles of *Holothuria* do not arise from the same radial canals as in *Cucumaria* is of great importance, but it is even more interesting to note that tentacles 6-10 arise in *Holothuria* from the same radial vessels and in the same order that they do in *Cucumaria*, *Synaptula*, *Chiridota* and *Rhabdomolgus*. In other words, while tentacles 1-5 are not homologous in the three families concerned, the radial canals and tentacles 6-10 are.² It is further of interest to note as bearing on a possible asymmetrical holothurian ancestor, that in all these genera, whose development has been studied, the mid-ventral radius is precocious and develops its organs earlier than the others.

² On p. 222 of his paper, Edwards says: "While Clark does not suggest the homology, it is possible to regard these 'secondary outgrowths' as the last vestiges in the degeneration of the protoholothuroid radial canals." I beg to call attention to the following passages from my memoir: "the second series corresponds to those which in *S. digitata* give rise to the radial water-canals" (p. 63); "the secondary outgrowths of the hydrocoel ring in Synaptidae are homologous with the five outgrowths of the hydrocoel ring in the true holothurians" (p. 69). Further emphasis on the same point will be found at the bottom of p. 81. It is hardly fair, therefore, to say that I did not "suggest the homology."

From our present knowledge of holothurian development, certain facts seem to be definitely determined. Of these the most important is that the five primary outgrowths of the hydrocoel in the Synaptidae, which give rise to the five primary tentacles, apparently have no homologs in the pedate holothurians. Associated with this is the fact that the secondary outgrowths of the hydrocoel in the Synaptidae are homologous with the radial canals of the pedate holothurians. These outgrowths (= radial canals), moreover, give rise to tentacles 6-10, in the same order and from the same radii, in all holothurians. Furthermore, in *Synaptula*, tentacles 11-13 also arise from these secondary outgrowths, and it seems to me probable that in synaptids with 15 or more tentacles, the additional ones arise from the same outgrowths too. As a corollary to these facts we are forced to conclude that the first five tentacles of *Cucumaria* and *Holothuria* have no homologs in the synaptids. It is, of course, possible to imagine that through some kind of inexplicable shifting, the primary interradial tentacles of the synaptids have been moved laterally and then outwardly on to the radial canals in the pedate holothurians, and thus to maintain that the primary tentacles are homologous in the two groups. There is no evidence, however, of such movement and it seems hardly probable; but it is clearly not impossible.

Some recent writers (Perrier, MacBride, Östergren) have seemed to minimize the essential difference between the Synaptidae and the other holothurians as revealed by their development, but in my judgment this difference far outweighs any of the morphological resemblances between the two groups. The unique character of the five primary tentacles of the synaptids is so remarkable that Ludwig's classification, by which the *Holothurioidea* are divided into the two subclasses *Actinopoda* and *Paractinopoda*, ought to be accepted. Since it is the first five outgrowths of the hydrocoel which give rise to the primary tentacles of the synaptids, while the secondary outgrowths (= radial canals) only appear subsequently, it seems to me we must suppose that interradial outgrowths (tentacles?) preceded radial canals in the development of the water-vascular system and that we must regard the *Paractinopoda* as the older group. The persistence of

primary tentacles and the complete degeneration of the radial canals in the adult, in addition to the entire loss of pedicels, seems to me to confirm this view. In 1898, I pointed out that there were three possible views regarding the relationship of the synaptids to the other holothurians: (1) that the synaptids are the primitive stock of the class; (2) that the synaptids represent a more primitive branch of echinoderms than that of the pedate holothurians; (3) that the synaptids are degenerate pedate holothurians. At that time I held to the third view. The works of Becher and Edwards, taken in connection with my study of *Cirridota*, lead me to believe now that the second view, which was first proposed by Cuénot ('91), is quite as near the truth. While I do not think it can be questioned that the synaptids are degenerate, pedate holothurians, I am satisfied that the pedate form from which they arose (the "Urholothurie" of Ludwig, "Stammholothurie" of Östergren) differed in certain very important points from any known form. I picture it as a short, thick-bodied creature with five stout, simple, interradial tentacles, much like a *pentactula* in form and moving like it, chiefly by means of the tentacles. Unlike the *pentactula*, however, it had well-developed radial water-canals with each of which was associated a double series of pedicels. From such an ancestor the *Paraactinopoda* have arisen, losing the pedicels and radial canals, while the *Actinopoda* have developed with the loss (or extraordinary shifting) of the five primary tentacles. Before the separation of the two sub-classes certain pedicels close to the circular water-canal became modified into tentacles, and in the *Actinopoda*, five of these (one in each interradius) seem to have replaced the primary tentacles. If Edwards' and Ludwig's observations are both correct, the same radial canals did not supply these five "pedicel-tentacles" in the two groups, *Aspidochirota* and *Dendrochirota*, and this would seem to indicate the separation of these two groups while this character was still unfixed. The origin of tentacles 6-10, curiously enough, appears to have been fixed for all three groups at an earlier period. At what point the *Elasipoda* began their divergence it is hard to say, but it may have been about the same time as the separation of the *Aspidochirota* and *Dendrochirota*. There can be little doubt that the *Molpadonia* were

derived from the *Dendrochirota* at a comparatively recent date and there is good reason for thinking *Eupyrgus* has had a different origin from the rest of the order and has simply converged into the group.

The relationship of the Holothurioidea to other Echinoderms

If the proposition made above, that the five interradiat tentacles of the synaptid pentactula are characteristic organs of the ancestral holothurian, be accepted, the first question which naturally arises, when we consider what relation the holothurians bear to other Echinoderms, is this: What interradiat outgrowths of the hydrocoel are there in other Echinoderms with which these tentacles can be homologized? At first approach the problem seems a very difficult one, for so far as our knowledge of echinoderm embryology goes, the only direct outgrowths of the hydrocoel in the other classes are the radial canals, pore-canal and polian vessels, all of which are also present in our hypothetical holothurian ancestor. A careful study of recent work of Grave ('02) and MacBride ('03) on the development of echini, has led me to the rather surprising conclusion that *the primary tentacles of synaptids are homologous with the dental sacs of the echini*. This idea at first seems preposterous because the dental sacs of echini have no connection whatever with the hydrocoel. More careful consideration shows, however, that this objection is far from conclusive. Both Grave and MacBride show that the dental-sacs of echini arise as five interradiat outgrowths of the left coelomic pouch, which lies below (or on the inner side of) the hydrocoel and from which the hydrocoel itself arose. These outgrowths push out between the budding radial canals, and thus these two series of outgrowths come to occupy the same position with relation to each other which the primary and secondary outgrowths of the hydrocoel occupy in the pentactula. There is, of course, this important difference that in the young echinoid, the lumens of the dental sacs have no connection with the water-vascular system, while in the pentactula the homologous lumens are continuous with that of the circular water-canal. If, however, the essential identity of the left hydrocoel and the left posterior coelom

in the echinoid larva be admitted the importance of this difference is somewhat diminished, since the lumens of the dental sacs are for sometime continuous with that of the left posterior coelom. There is, moreover, another feature in the development of the young echinoid, which is strikingly like one shown in the development of the pentactula. This is the invagination of an ectodermal plate, forming the so-called "amniotic cavity," from the floor of which the oral disc of the sea-urchin arises. In all essentials this process is similar to the formation of the "atrium" in the development of the pentactula, and the relation of the radial canals and dental sacs to the floor of the amniotic cavity is identical with that of the primary and secondary outgrowths of the hydrocoel to the floor of the atrium.

If the homology of parts, between the young echinoid and the pentactula, here suggested, be accepted, we have new proof of the close relationship of holothurians and echini. The presence of jaws (and hence of dental-sacs) as a fundamental character of echini has recently received remarkable confirmation by Mr. Agassiz's announcement ('09) that in the very young *Echinoneus* (a simply organized spatangoid) a complete set of jaws is developed only to be rapidly resorbed. The presence of five conspicuous interambulacral tentacles as a fundamental character of para-actinopod holothurians is confirmed by the observations on *Chiridota*, published herewith. The assumption that these two sets of structures are homologous is purely hypothetical but seems to me justified by the facts already known.

Granting this hypothesis, it follows naturally that in the probable ancestor of both echini and holothurians, the separation of the hydrocoel from the left coelomic pouch did not take place until the outgrowths for the radial water-vessels and, alternating with them, the rudiments of five primary tentacles, had been formed. In other words, the water-vascular system was not a closed system but was a part of the body cavity, and the lumens of both radial vessels and tentacles were continuous with the body cavity. In echini, however, owing to the formation of heavy calcareous deposits (teeth) in these primary tentacles, their development has been greatly retarded, and the hydrocoel forms and separates from the rest of the coelom before they arise. In

paractinopod holothurians, on the other hand, the development of the primary tentacles has been accelerated by their usefulness as tactile and locomotor organs, and, therefore, they have come to surpass the radial water vessels, not only in size but in their earlier and more rapid development.

It would be easy to draw a picture of the hypothetical echino-holothurian ancestor in accordance with the theory here proposed, but it could hardly be worth while. Suffice it to point out that the five primary interradian tentacles of such an ancestor doubtless assisted in the pushing of food into the mouth, and because of this use two very different lines of development opened up. Along one line, the tactile function was specialized and the organs became delicate, sensory tentacles, while along the other line, the mechanical function of scraping food, from the surface on which the animal crept, was specialized and the organs became hard, non-sensory teeth, accompanied by the necessary muscles and calcareous supports. The loss of a hard dermal skeleton in the one case, and its perfected development in the other, were naturally correlated accompaniments. In accordance with this theory, it may be possible to homologize the perignathic girdle of echini and the calcareous ring of holothurians. I am inclined to think, however, that such a homology does not exist. But it does seem to me probable that there is a true homology between the radially placed sphaeridia of echini and the paired positional organs, accompanying the radial nerves of synaptids. At present, however, I have no evidence to offer bearing on the point.

SUMMARY

1. Östergren's view that the ancestral holothurian was a mud-loving Clasiopod-like form is rejected for three reasons: (1) Too little weight is given to the difference between primary and secondary simplicity of structure; (2) The ancestral holothurian was probably not a mud-loving but a plankton-feeding^{*} form; (3)

^{*} By "plankton-feeding form," is meant an animal that lives on micro-organisms which it takes from the water about it. In this case, I would include also the gathering of micro-organisms from the rocks or hard bottom on which the ancestral form lived.

Deep-sea animals, such as the *Elasipoda*, are, as a rule, highly specialized forms.

2. Becher's work has shown that *Rhabdomolgus* is essentially a synaptid in structure and development.

3. Edwards' work has shown that tentacles 1-5 in *Holothuria* are not homologous with tentacles 1-5 *Cucumaria*, but that tentacles 6-10 are homologous in *Holothuria*, *Cucumaria* and the *Synaptidae*.

4. Ludwig's division of the *Holothurioidea* into two sub-classes, *Actinopoda* and *Paractinopoda*, is accepted, since tentacles 1-5 of the *Synaptidae* are interradial outgrowths of the hydrocoel, and have no homologs in the other holothurians, while tentacles 1-5 of the pedate holothurians have no homologs in the *Synaptidae*.

5. Since the primary outgrowths of the hydrocoel in synaptids develop into organs lacking in the *Actinopoda*, while the secondary outgrowths correspond to the actinopod's primary hydrocoel outgrowths, it would seem that the *Synaptidae* are an older and more primitive stock.

6. The ancestral holothurian is pictured as a pentactula-like animal, but provided with radial water-vessels, accompanied by double series of pedicels.

7. The *Paractinopoda* have arisen from such a form with retention of the primary tentacles and loss of radial water-vessels and pedicels. The *Actinopoda* have developed the radial water-system and lost the primary tentacles.

8. The five primary tentacles of the *Synaptidae* are possibly homologous with the dental-sacs of echini.

9. The "atrium" of the pentactula is possibly homologous with the "amniotic cavity" of echini.

10. In synaptids the tactile function of the tentacles has been specialized to a high degree, while in echini, the homologous organs have, through the mechanical function of scraping, been developed into teeth.⁴

⁴ I trust this statement will not be taken at its face value, as pure Lamarckianism. I personally feel no doubt that natural selection, acting on slight variations, has been the real agent at work.

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PLATE 1

EXPLANATION OF FIGURES

1 Old gastrula of Chiridota. $\times 70$. *ar* = archenteron; *bl* = blastopore; *hy-en* = hydro-enterocoel.

2 Young larva of Chiridota. $\times 70$. *ac* = anterior coelom; *ar* = archenteron; *bl* = blastopore; *cb* = ciliated bands; *cp* = calcareous particles; *hy* = hydrocoel; *len* = left enterocoel; *ren* = right enterocoel; *wp* = water-pore. The hydrocoel already shows the five primary outgrowths and even the secondary ones are indicated.

3 Very young pentaetula of Chiridota. $\times 70$. *ac* = anterior coelom; *al* = alimentary canal; *bc* = coelom, *cb* = ciliated bands; *cp* = calcareous particles; *ht* = primary tentacles; *pv* = polian vessel; *wp* = water-pore.

4 Pentaetula of Chiridota. $\times 70$. *a* = anus; *ac* = anterior coelom; *atc* = atrial collar; *cb* = ciliated bands; *cp* = calcareous particles; *cr* = calcareous ring; *i* = intestine; *pt* = primary tentacles; *pv* = polian vessel; *rn* = radial nerve; *so* = sense-organ; *st* = stomach.

5 Figures showing the development of the trochoderma-like wheels (*f*) from the simple discs (*a*) $\times 310$.

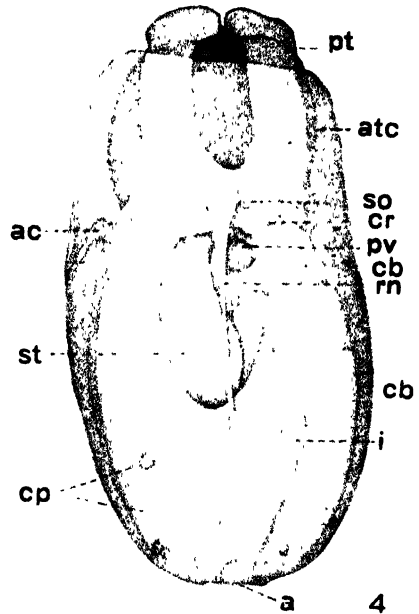
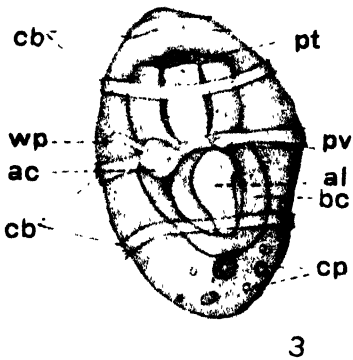
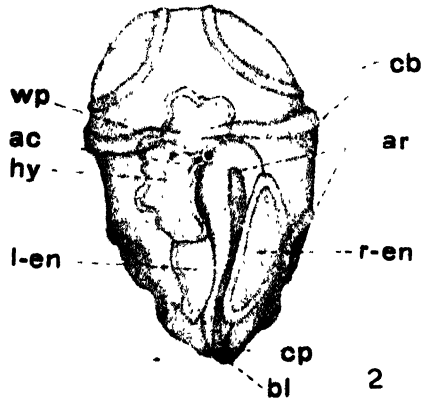
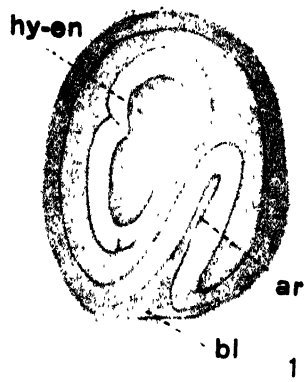
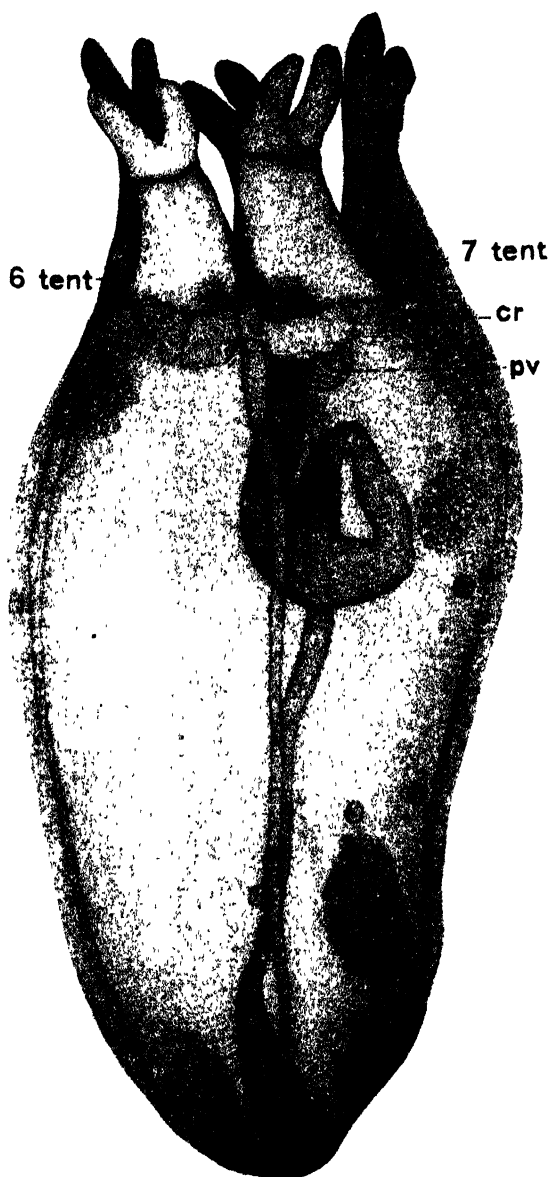


PLATE 2

EXPLANATION OF FIGURE

6 Seven-tentacled young of *Chiridota*, seen from the ventral side. $\times 70$. *cr* = calcareous ring; *pv* = polian vessel. The beginnings of the sixth and seventh tentacles are shown, and the heaps of six-spoked wheels are conspicuous. The wheels with numerous spokes are still common, however.



6 tent

7 tent

cr

pv

ON THE STRUCTURE OF CRYPTOGONIMUS (NOV. GEN.) CHYLI (N. SP.), AN ABERRANT DISTOME, FROM FISHES OF MICHIGAN AND NEW YORK

HENRY LESLIE OSBORN

Professor of Biology and Geology, Hamline University

SEVEN FIGURES

DISTRIBUTION AND HABITS

The material from which the present article has been prepared was derived in part from fishes taken in Lake Chautauqua, New York, and in part from others from St. Mary's River, Michigan, a river connecting Lakes Superior and Huron. The worm has been found chiefly in the stomach and intestine of the black bass, *Micropterus dolomieu*, where it is found disseminated through the creamy chyle. The minute worms are detected as black spots of elongate form. They are imbedded in the layer of chyle itself and not attached to the wall of the digestive organ of the host, which seems strange especially in view of the size of the suckers which are unusually well developed. The particular localities from which the worms were derived were near the grounds of the Chautauqua Assembly at the head of the Lake, and in St. Mary's River in the narrow passage between the small island of Neebish and the upper end of St. Joseph's Island. A brief preliminary description of the worm appeared in the *Zoologischer Anzeiger* ('03). Since that time the worm has been reported by Stafford ('05) from the rock bass (*Ambloplites rupestris*) in Canadian waters. The occurrence of the worm in these two localities is interesting, for while the Michigan and Canadian situations are a part of one river system the Chautauqua location is not connected with them and is a part of the Mississippi River

system, Lake Chautauqua being at the head of one of the tributaries of the Ohio River.

The worm has also been recognized by me in the rock bass from Lake Chautauqua occurring in the same way as in the black bass. I have also at Lake Chautauqua found that many of the small fishes are more or less infected with small black spots in the skin, both of the general surface and especially in the membrane on the fins. In one instance the pectoral fin of a small sun fish exhibited a small white cyst in the membrane between the fin rays. A drawing of this was made at the time and from this fig. 7 has been copied. It contained a living immature individual which is adequately shown by its two ventral suckers to be identical with the species now under consideration.

In the chyle in which this worm was found there are generally two other distomes: a species of *Bunodera* since described as new and designated *B. cornutum* (Osborn '02) and an immature and undetermined fluke whose genital organs are sufficiently developed to locate the reproductive opening at the posterior end, thus placing it with the *Urogonominae*. These latter were traced back to black colored cysts found in the fins and skin of smaller fishes. In the masses of slime in which *Cryptogonimus* and these other flukes occurred, I found in one instance the remains of vertebral centra of a small fish. This justifies the inference that the worm reaches the bass, its definitive host a predaceous fish, by way of the smaller fishes, and suggests that the search for the unknown primary host of *Cryptogonimus* will have to be made among the animals which serve as food for the small fishes.

My notes of observation on the living worms contain very little on outward movements. They were removed from the chyle in which they were found and watched in salt solution. Under this condition many flukes are very active. I have given elsewhere ('04) an account of the movements of *Cotylaspis insignis*. *Cryptogonimus* under this condition lies nearly motionless with the ventral surface up most of the time. Occasionally the worm turned over but soon returned to its inverted position. The only general bodily movements were an elongation of the anterior end and its retraction, but no movements of locomotion were

seen. The oral sucker was kept commonly in a state of partial contraction. In some cases it was seen to be employed in the act of adhesion. The ventral suckers, especially the anterior one, occasionally were seen to be somewhat extended beyond the contour line of the body wall and then withdrawn.

METHODS

Careful studies of the internal organization of the living material were made, with especial efforts to determine the anatomy of the excretory system, since no other method demonstrates the minute parts of this system as well. A certain degree of compression which can be readily regulated by absorbing the water by means of little wedges of bibulous paper is favorable. After trematodes have been confined for some time the tissues become clearer and organs previously invisible can be seen. Many of the worms were separated out from the chyle and killed and fixed in aqueous corrosive sublimate solution. Some of these were stained in borax carmine and mounted whole in xylol balsam, under a cover glass supported on rollers, made by drawing melted glass tubing out into threads. This method permits one to roll the animal over and view it in every position, and was found very useful. It is, however, to serial sections that we must turn for the most precise information of the organization of this small worm. Its minute size made it necessary to devise a method of imbedding in which records of the orientation were kept. This was done by pouring melted paraffine into a cold watch-glass under the simple microscope and then adding from a warmed pipette a drop of melted paraffine containing one of the infiltrated worms. As the worm gradually sank into the stratum of half congealed paraffine on the bottom of the watch-glass it could readily be adjusted in any desired position with a heated needle and held there a moment till the paraffine was cold enough to hold it in place. A mark on the surface of the paraffine sufficed to show the position of the worm within. Series were cut in the usual planes and stained on the slide in iron-haematoxylin. This reagent produces very fine anatomical, and in some respects, cytological re-

sults. The sections are over-stained and the color is drawn under low magnification and can thus be checked very satisfactorily.

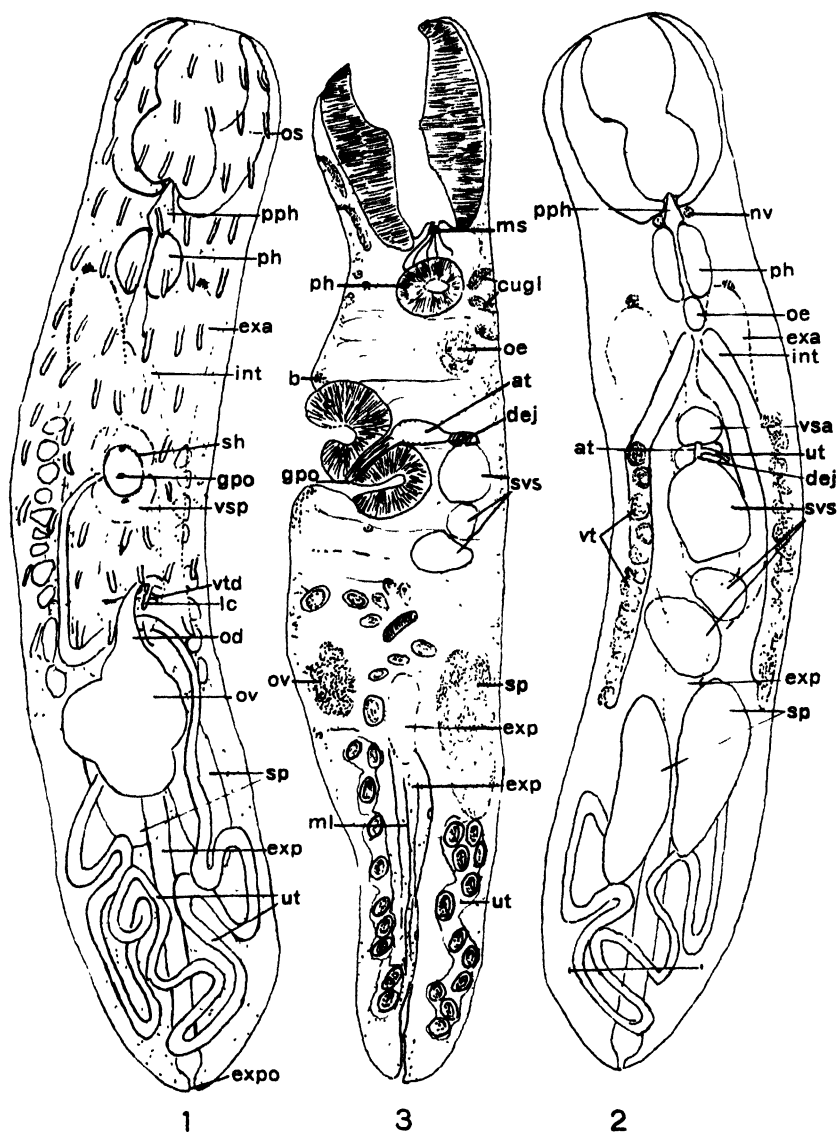
EXTERNAL FEATURES

This is one of the smallest distomes. Eighteen specimens, after mounting in balsam, show a variation in length ranging between 0.525 mm. and 1.3 mm. The length of four other micro-trematodes is stated to be: *Gymnophallus somateria* 0.5 mm. (Odhner '00), *Levinsenia pygmaea* from 0.42 to 0.60 mm. (Jagerskjold '00), *Distomom confusum* and *D. perlatum* 1.3 mm. (Looss '94). The shape of the body, seen by a comparison of figs. 1, 2, 3, and 4, is somewhat tubular, the anterior third is somewhat flattened and the rest cylindrical. In the anterior level the diameters of the body are 0.155 mm. and 0.09 mm. Posteriorly the body is slightly compressed, measuring 0.15 mm. by 0.13 mm. (fig. 3). The enlarged proportion of the body in the middle region is easily seen to be directly correlated with the locations of the members of the reproductive apparatus.

The oral sucker is very large and prominent relatively to the total size and much larger than the ventral suckers. It nearly fills the anterior end of the body and its length is contained about five times in the total body length.

In most cases its outline has the form shown in figs. 1, 2 and 3. It measures .014 mm. by 0.12 mm. Its cavity is subdivided by a constriction near the centre which shows distinctly both in total preparations and sections (see fig. 3). The wall of the anterior part of this cavity is sometimes widely opened giving the sucker a very flaring funnel shape. A small percentage of my preparations have the oral sucker in this shape but the shape in fig. 1 seems to be the resting form of the organ.

In the position usually occupied by the ventral sucker there is an adhesion apparatus which involves several features entirely unlike anything thus far known elsewhere among trematodes. It consists of a chamber with a mouth opening controlled by a sphincter muscle (*b* in fig. 3). Located in this chamber there



1 and 2 Ventral and dorsal views of *C. chili*, based on a total preparation in xylol balsam, after corrosive fixation and borax carmine staining. The spines have been omitted and the coils of the uterus simplified for clearness. $\times 18$ diameter.

3 Longitudinal section in the sagittal plane, drawn by combining parts from three different sections of the same series, into a single view.

are two muscular organs, each one possessing the structure found in the suckers of distomes. These relations are shown in fig. 3, also in greater detail in fig. 5. The suckers are not, strictly speaking, external organs in this case. A more detailed account of them will be given below in connection with the accounts of the internal organization.

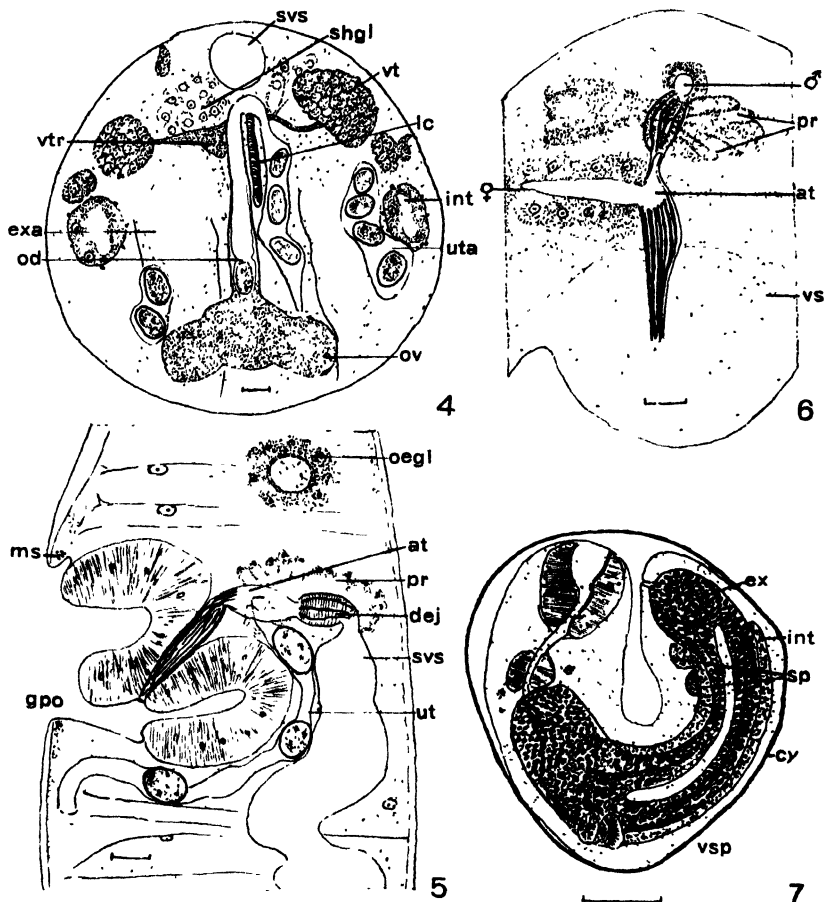
The genital opening is located inside this sheath in the mid line of the body between the two ventral suckers, (see fig. 1, *gpo*, also fig. 3). In the latter the course of the outer end of the genital duct is shown running down between the two suckers.

The excretory opening is located at the extreme posterior end of the body as usual.

The body wall is extremely spinous throughout. The spines are very conspicuous in living specimens viewed in favorable lights, and give the animal almost a shaggy appearance. They are so broad in their anterior margins that they may almost be designated scales, each one has a short basal piece inserted in the cuticle carrying an outer triangular portion whose base lies in the transverse axis of the body. The spines lose their broadened form posteriorly and have more the hook form, as in trematodes at large. They are deeply stained in all iron haematoxylin preparations while the cuticle is entirely uncolored.

There are certain internal structures attached to the body wall by one end and extending freely posteriorly from this point of attachment. Following the designation of other writers for similar structures found elsewhere in this group (*e.g.*, *D*, *clavigerum*, [Looss '94] fig. 30) I will call these organs glands. In life they can be seen as highly refractive objects swaying to and fro with the movements of the body, evidently entirely free except at the cuticle connection. These glands (shown in fig. 1) lie in transverse rows girdling the body and extending only as far back as the level of the beginning of the posterior third of the animal. In sections these glands display their attachment at the surface and the rest of the gland extends freely into the interior of the body.

The body wall musculature is rather feebly developed. In worms mounted whole under most favorable illumination the



4 A slightly schematic cross-section, combining the anatomical features of three successive cross-sections, taken on the level of the origin of the oviduct from the ovary. Scale = 0.01 mm. — $\times 350$.

5 A somewhat idealized view to show the construction of the ventral sucker apparatus and the terminations of the genital ducts. The drawing is based on the series from which fig. 5 was drawn and given more depth than that of the section. As a fact the uterus and the prostate gland are not in the median plane while the ductus ejaculatorius and atrium are, $\times 500$

6 A still more diagrammatic view of the terminations of the genital ducts all the parts being shown as if in the same plane in a transverse section $\times 500$.

7 A whitish, globular cyst from the pectoral fin of a sun-fish from Chautauqua Lake, New York, containing an immature specimen of *C. chyli*. The worm is seen ventrally. The intestinal coeca are filled with highly refractive droplets, beneath them the excretory chambers appear filled with globular bodies. Scale, 0.1 mm $\times 100$

usual longitudinal circular and oblique fibres can be seen. In tangential sections the fibres seen are scanty and very small.

On the other hand the suckers have more than the usual size. The dimensions of the oral sucker have already been noted. Its histological structure has the usual characteristics, the mass of the organ being a very thick wall made up principally of radial muscle fibres among which at wide intervals there are a few nuclei.

The ventral suckers show well in figs. 1, 3, and 5. They are of the same size and lie in the median plane. The anterior one is more ventral and nearer to the surface of the animal, it is also more nearly opposite the opening of the sheath, as shown in fig. 3. In fig. 1 the lip of the sheath can be seen cutting across the outlines of both suckers. Posteriorly the body wall folds in a considerable distance to reach the posterior sucker as in fig. 5.

In addition to the possession of two ventral suckers of equal value 'this apparatus is still further aberrant, inasmuch as it is a semi-internal organ, made so by the development of a sheath or pocket into which the suckers are withdrawn, so as to be wholly immersed within the contour of the surface, where they can be partly enclosed by a sphincter muscle located in the margin of the sheath. In living worms the lips of this sheath are at times considerably dilated and the suckers exerted, at other times the lips are contracted. The mechanism of this movement seen in section consists of a band of muscular fibres shown at *d* in fig. 3 and *ms* in fig. 5. This sheath is a totally unique anatomical feature as well as the two ventral suckers.

So far as I am aware, such a peculiarly constructed ventral sucker apparatus is quite unique in distomes. A second case of a fluke with two ventral suckers has been reported, that of *Podocotyle* (Luehe, '00) but the structure of that form is entirely different from this, one of the suckers being located on a long stalk and there being no sheath. In no other instance is there such a sheath with sphincter muscle lodging a second fully developed sucker. There are no data at hand by which to decide which of the ventral suckers should be homologized with the ven-

tral sucker of the group generally. From its position the anterior one would seem more probable, since it is more ventral and nearer the surface. But against this view must be noted the position of the genital opening which is usually in front of the ventral sucker and rarely if ever behind it when in such close relation with it. The anatomical situation in regard to these points is decidedly novel and aberrant. In the total absence of embryological information and of any closely related forms we shall be forced to leave the question of homology unsettled.

NERVOUS SYSTEM

The faintly colored minutely granular matter seen in trematodes, generally in the chief nervous ganglia, is seen here in sections in the position indicated by the letters *nv* in fig. 2 and in the whole worm masses can be seen in this position which are plainly the chief nerve mass. In sections nerve cell bodies can be seen on the borders of these masses.

In whole worms a black spot is very distinctly visible on each side of the body on the level of the pharynx. Figs. 1-2 show its position. They are encountered in the transverse sections where they are seen to be very deeply seated. The organ is shown by immersion objective to be composed of small rounded grains of deep brown color, held together in a mass of oval outline, but no special cells can be seen in connection with them. They are constant in position when present, but in a few individuals they are wanting, and in some, one is present and the other is wanting. Eyes which are present in early stages of trematodes are wanting in the adult stages as a rule (though with numerous exceptions, *e.g.*, *Cotylaspis*, Osborn, '04). In favor of considering these pigment masses eyes is the fact of their position; their structure does not offer favorable evidence nor their absence in some cases. It is, however, possible to regard them as degenerate organs of vision, vestigial structures possibly. No traces of other sense organs were found.

ALIMENTARY SYSTEM

The space between the oral sucker and the pharynx is occupied by a prepharynx, figs. 1, 2. This is a very thin walled tube thrown into many different shapes in accord with the contractions of the animal, connecting between the cavities of the oral sucker and the pharynx. In many cases a small conical point projects forward into the base of the cavity of the oral sucker (figs. 1 and 2). At this part the wall is furnished with circular fibres (*ms*) which act as a sphincter. Behind these the wall is supplied with longitudinal muscles. When fully extended the prepharynx has a length of about 0.06 mm.

The pharynx is well seen in the surface views in figs. 1 and 2. In the whole animal from which fig. 1 was drawn there seems to have been no retraction of the anterior end, such as is seen in many of the preparations. In fig. 3 the organ lies transversely to the axis of the body and is less normal than the position in fig. 1. The pharynx measures 0.7 mm. in length. It is somewhat flattened from side to side measuring 0.03 mm. and 0.07 mm. in its lesser and greater diameters. Its wall has the usual muscular structure.

The oesophagus is surrounded by a layer of cells. They are very indistinct in all my preparations but apparently they are 0.01 mm. in length and pyriform tapering toward the passage. Their deeper ends form a somewhat distinct boundary for the mass, in which the rounded ends of the cells can be seen, lodging nuclei of a constant size. These have a sharp membrane and a large and distinct nucleolus, and are clear and nearly devoid of chromatin. The cells in the parts nearer the lumen of the oesophagus lose their boundaries and the mode of connection with the oesophagus cannot be seen.

Cells are found in this position in many trematodes and are often referred to as salivary glands. Otto ('96) calls attention to a view of Leukart's that they are more likely to be parenchyma cells employed in the secretion of the cuticle, and favors this view. Notwithstanding the absence of physiological and histological evidence it still seems best to designate these cells oesophageal glands.

The posterior end of the oesophagus lies immediately under the dorsal surface. The two intestinal coeca arise directly from it and pass obliquely backward as shown in fig. 2. They run downward as far as the vitellaria, not passing into the posterior end of the animal. In the encysted stage (see fig. 7) they are relatively longer and reach almost to the posterior end. Most of the intestinal coeca lies in the level indicated in the schematic cross section, fig. 4. Each coecum is a strictly simple tube, whose epithelium is low and flat as shown both by the boundary line of the inner wall and by the widely separate nuclei. In no case have I seen any tall epithelium cells in the intestinal epithelium or any boundaries between the cells.

Favorable longitudinal sections in several different series show a longitudinal musculature in the wall of the intestine. This is composed of a very few widely separated fibres, fig. 4. Such fibres can be recognized in all parts of the organ but in no case has it been possible to demonstrate the presence of any circular fibres.

Usually no food was visible in the cavities of the alimentary organs, but in some worms the oral sucker and prepharynx were filled with minute granular material which, under the highest magnification and favorable illumination, proved to be made up throughout of objects which, when stained with borax carmine, present a flat rounded unstained portion lodging a deeply stained nucleus or a mass of small deeply stained particles which can be considered a disintegrated one. Though I have no complete proof to present, there seems little reason to doubt that these are blood corpuscles. In the encysted specimens from which fig. 7 was drawn the intestine was filled as far forward as the level of the ventral sucker from the posterior end with small rounded objects.

EXCRETORY SYSTEM

The excretory system, so far as I have succeeded in determining its anatomical structure, is exceedingly simple. There is a terminal pore, fig. 3, located as usual, from which a slender short passage runs forward to reach the end of a rather large posterior

excretory chamber (*exp.* of fig. 3). This is seen in all the cross sections occupying a position in the centre of the section, it is of varying diameter and in places measures 0.05 mm., equalling fully a third of the entire diameter of the body at the same level. That this chamber is not merely a space among the other organs is clearly indicated by the existence of a definite wall, seen in sections as a thin sharp line, in which nuclei are distinctly recognizable, and of which surface views are also obtainable. Fig. 3 includes a view of the organ seen on its surface posteriorly and in its lumen anteriorly. The boundaries of cells cannot be found in the wall and the line is not a double one but the relation of the nuclei warrants the belief that a definite cellular wall exists. In surface views the wall is a faintly stained finely grained film. In this film there are a few widely separated muscle fibres (*ml* in fig. 3), which are very clearly a part of this organ. Moreover, the living organ possesses the power of contraction. In living adults under observation I have seen pulsations taking place in this wall and discharges of granular material passing out of the terminal pore.

Anteriorly on the level of the front of the ovary this tube forks where it reaches the bar formed by the oviduct and the two branches pass on forward till they reach the level of the pharynx. In living worms these branches are very conspicuous as clear spaces much lighter than the surrounding organs. I have tried to give this effect in figs. 1 and 2. In life these excretory cavities possess a smooth surface and, seen in profile, a sharp boundary. This makes it look as if there were a definite wall, but in views on sectioned material a wall cannot be recognized. In sections which show such clear evidence of a structural wall in the posterior cavity, as we have in fig. 3, there is no evidence of a similar wall or of any wall at all in the anterior cavities. The conclusion which we are forced to draw from this fact is that the anterior cavities are merely intercellular spaces permanent in position but not supplied with a definite wall of their own. The outer parts of the excretory system are thus more highly developed than the inner parts: or the anterior may be considered more embryonic than the posterior.

I have made every effort to determine whether the usual flame cells and capillaries are present here or not. No traces of either have been seen. This negative evidence does not suffice fully to demonstrate the absence of flame cells but the excretory capillaries and tubes of trematodes even of quite immature stages are usually very distinct structures and readily recognized so that it seems as if they would have been seen in this species if they are in existence. It seems most likely that they are not developed at all and that if the flame cells are in existence their products are merely conveyed by way of spaces among the cells to the nearest point in the system of excretory cavities.

In all adult cases examined, whether of living worms or of total mounts or sections, the cavities of the excretory apparatus were found entirely empty. As already stated, contractions of the wall and the escape of excretory substance were observed. This was in marked contrast with the conditions in the encysted stage, in which (see fig. 7) the excretory apparatus is somewhat as in the adult, except for the confluence of the anterior cavities at the front level. In this stage the cavities were filled with small very highly refractive globules. Each showed a very refractive surface contour and clear interior like an oil droplet. They were of different sizes and each kept its identity. I have found a similar condition in *Clinostomum* where in encysted worms the excretory cavities were filled with such granules which were at once discharged on liberation of the worm from the cyst. It would appear from these facts that the process is one of storage during encystment as a mode of disposing of the waste products pending the liberation of the worm.

It is seen from this account that the excretory system is much less differentiated here than is usual in flat worms. I am inclined to regard this condition as an arrest in the embryonic development of the system, while the other systems of the animal have gone on in the usual manner. In casting about for a cause to which to attribute the supposed arrest the most probable fact is the small size of the animal. The amount of waste chemical material must be very small, and such a very elaborate and extensive apparatus for excretion as the one found for instance in

Cotylaspis insignis (Osborn '04) would be a quite unnecessary luxury. It would be quite easy to imagine that in *Cryptogonimus* the wastes of the body find their way into the large anterior cavities and thence to the posterior cavity. We must suppose that *Cryptogonimus* is descended from large sized ancestors and that its diminutive dimensions are secondary, just as we should suppose in the case of *Cyclops* among the crustacea and *Diemyctylus* among the urodeles, and we are justified in recognizing simplicity in organization as possible and adaptive in all these cases, as it evidently is in the case of the lungless salamanders.

REPRODUCTIVE SYSTEM

The ground plan of the reproductive apparatus is shown in fig. 1. The parts of the apparatus are confined to the middle and last thirds of the body. The spermaries and ovary are opposite, the former being dorsal and the latter ventral. There is a very large seminal vesicle, no cirrus organ, a small ejaculatory duct. The terminal passage common to the male and female apparatus, opens to the exterior between the two ventral suckers. The coils of the uterus are confined entirely to the hinder body-third. No Laurer canal opening has been recognized. The vitellaria are composed of numerous small glands restricted in location to the central regions of the body.

The spermaries, fig. 2, lie almost on the same level directly below the dorsal body wall. Fig. 2 shows that one is placed on the left side and the other on the right, and the right is slightly in advance of the left. They are oval and compact, measure 0.2 mm. and 0.3 mm., respectively. No cellular wall can be seen in any of my sections. Distomes differ in this point, in some, *e.g.*, *Cotylaspis* (Osborn, '04, fig. 38), a wall of nucleated cells can be distinctly seen. A surface layer of cells "parietal cells" can be seen in sections while the interior is made up of cells showing more or less evidence of activity in the direction of spermatogenesis. The parietal cells are somewhat smaller than the central cells, each one possesses a relatively large nucleus which is very granular and deeply stained. The central cells are larger than the pari-

etal cells, measuring 0.006 mm., and have a very large nucleus (0.005 mm.) with membranes, granular chromatin and a large and distinct nucleolus, or in some cases the nuclear membrane is wanting and the chromatin gives indications of activity, in one case radially arranged cells are present but in the main the material is not favorable for study of spermatogenesis.

It has not been possible to recognize the ducts leading from the spermaries to the very large seminal vesicle, either in living specimens or in preparations. The seminal vesicle is a very large organ occupying the dorsal region of the body in the space between the spermaries and ventral suckers. Figs. 2, 3 and 5 show its position. It has a winding course which produces the broken effect shown in figs. 2 and 3. The diameter of the organ varies somewhat, it is about 0.05 mm. or less and is nearly uniform throughout. The wall is a thin sharp line, in which in places indications of circular muscle fibres are present. The organ is filled with spermatozoa in all cases which have come under my notice, showing that the spermaries must have been active recently.

At its anterior end the cavity of the seminal vesicle suddenly narrows into a tube of a diameter of 0.01 mm. which bends sharply at a right angle with the seminal vesicle and passes in a ventral direction. This passage may be called the ejaculatory duct since it is surrounded by the prostate cells. It measures 0.02 mm. in length and 0.01 mm. across. Its wall is somewhat muscular. There are a few longitudinal fibres and a few circular ones; neither lie close enough together to make a continuous sheet of muscle. Figs. 5 and 6 show this duct and 6 gives a fairly accurate view of the musculature fully corroborated by longitudinal sections of the animal. Anteriorly the ductus ejaculatorius passes into a chamber of the same size with which the uterus communicates and which may hence be called the atrium.

Surrounding the ductus there are certain peculiar cells shown in the diagram, fig. 5. These cells are confined to this part of the body. They lie in a loose and open mass on each side of the ductus. In a cross section of the animal they show an arrangement as if radiating from the ductus. These cells present a globular free end in which a nucleus is seen surrounded by a small amount

of cytoplasm and as much of the cell is occupied by a large clear space, the cells have much the appearance of mucous cells. There seems to be no objection to identifying these cells as the prostate gland.

The atrium (see fig. 3) has about the size of one of the ova. Its wall is different from that of the ductus in being less muscular. It receives the uterus by opening on its right side. It is continued by a slender passage which runs obliquely in the space between the two ventral suckers to reach the genital pore. Its wall is equipped with a close layer of strong longitudinal fibres, no circular fibres can be seen and they seem to be absent. In order to exclude the possibility that one of the ventral suckers might be a part of the genital system, which possibility is suggested by the existence of genital suckers in some trematode. (as for instance that of *Cladorchis*, Fiscoeder '03, fig. 80), a very careful examination of the exact relations of these terminal parts of the reproductive system was made, and all sections show with the utmost accord that the two ventral suckers are identical in structure and that neither one has any connection with the passages of the reproductive system.

It has also been possible to demonstrate the entire independence of these parts by physiological evidence. In living specimens under observation it was possible to follow eggs in their course as they travel down the uterus toward the exterior. Such eggs pass close to the ventral surface then move up and around the posterior sucker, then down between the two suckers and finally emerge between them.

There is a very great amount of difference among the trematodes as to the details of anatomical structure of the outer organs of the genital system. In the most highly developed terminal or cirrus organ a sack encloses: an eversible cirrus or penis, an ejaculatory duct and a passage still deeper surrounded by the prostate gland cells and behind these the seminal vesicles. Such a cirrus organ is present in *Clinostomum marginatum*, an account of the anatomy of which is now in course of preparation by the writer. A less highly developed stage of organization is that found in *Cotylaspis insignis* (Osborn '04, fig. 35) where a strong mus-

cular wall encloses the outer organs but does not include the seminal vesicle. And lastly there are cases like the one at present under consideration in which there is no development of a cirrus sack at all, so that here organization in this respect is at its minimum.

The ovary, as already noted and as shown in figs. 3 and 4, is close to the ventral surface of the body, and opposite to the spermaries. The organ measures 0.09 mm. in length and width, 0.05 in thickness and is lobed on the surface so as to be divided into two lateral portions and an anterior and posterior median portion. These indentations are however only a feature of the surface of the organ, the interior of the lobes being perfectly continuous.

The oviduct passes nearly directly across the body. Fig. 4 is a view showing this. It is based on three consecutive sections and is slightly diagrammatic. In one of these sections an egg happens to have been caught just at the entrance of the oviduct. This egg is already completely formed, the ovi-cell and vitelline cells are readily distinguished and the shell is present. There are certain cells present in the spaces around the oviduct. They are long and taper toward the beginning of the oviduct, and are doubtless the shell gland (*shgl* of fig. 4). The oviduct near the seminal vesicle bends suddenly upon itself and passes ventrally as the uterus on the left side of the oviduct. At this bend a passage can be seen in sections, to join the oviduct from which point of origin it runs ventrally between the oviduct and the uterus. The wall of this passage is well supplied with circular muscle fibres. It is parallel sided, can be followed in a few successive sections posteriorly and soon ends blindly. There are two organs in trematodes with which it is possible to connect this organ: one the seminal receptacle, the other the canal of Laurer. The form of the organ opposes its identification with a seminal receptacle which is of a saccular form and leaves us as the only alternative the canal of Laurer. That usually opens to the surface of the body dorsally, it also in some forms opens into the intestine. Both of these possibilities are excluded in this case as the tube is not running in either of these directions, when after having been

very conspicuous indeed it suddenly disappears from the series of sections, and we may suppose that it is here rudimentary.

A structure, (*vid* in fig. 4), can be seen running from the vitellaria toward the region of the oviduct with which the supposed canal of Laurer connects. This is filled with cells which are clearly yolk cells identifying the duct as the vitelline duct. On the right side a larger organ filled with cells is probably the yolk receptacle, their connection with the oviduct have not been recognized as yet.

The uterus takes the course indicated in fig. 1, in probably a majority of cases, but I have found some varying individuals in which it is reversed. Commonly it passes to the ventral surface on the right and with more windings than are shown in the drawing passes to the posterior end of the body. It then crosses to the left side and runs forward to the level of the ovary, crosses dorsally to its anterior end and runs forward on the ventral side till it reaches the level of the ventral suckers. Its course from this point is indicated in the semi-diagrammatic view, fig. 5.

The uterus in all cases contains some eggs in every part, as can be seen in figs. 3, 4 and 5, the older eggs are given a heavier outline and are darker. The eggs are not essentially larger in the ascending portion of the organ, and excepting for some loss in the distinctness of the ovicell in the older eggs there is no evidence of development in any cases which have come to my notice (in ten series of sections and more than twenty-five whole mounts, and very many living worms). The egg measures 0.01 mm. in the smaller and 0.02 mm in the greater diameter. There is an operculum. The older eggs have a very much thicker shell and so are much darker. In the whole animal they are black and they are responsible for the black spot in the chyle which shows the presence of the worm.

The vitellaria are confined to the central region of the body. They lie directly under the surface, externally to the excretory cavities and the intestine, their position is shown in fig. 4. There are numerous follicles measuring variously some 0.03 mm. across. The larger ones are often flattened somewhat. They are composed as usual of numerous yolk secreting cells.

CONCLUSION

It is not possible in the limits of this paper to make a study of the taxonomic relations of this worm. There is no sub-family in the scheme offered by Looss ('99) in which it can be located at all satisfactorily. Thus the Coenogonominae are small and scally, with genital pore close to the ventral sucker, male and female ducts united before they reach the surface, prostate free in the parenchyma at the end of the seminal vesicle, and no copulatory organ, all of which features are found in *Cryptogonimus*, but they have small neck, mobile body, testes elongate in transverse axis, receptaculum seminis large and resembling the ovary, uterus windings not passing behind the testis, and are found in the small intestine of warm blooded animals. Other assignments also offer difficulties. If we are guided by the simple condition of the copulatory organs with prostate free in the parenchyma and close relation of genital pore and ventral sucker as well as the presence of large anteriorly forked excretory cavities, then we are drawn toward *Microphallus* (Ward '94 and '01), though the anatomy in many points is so different, as in the small size of the oral sucker, the rudimentary state of the intestine, presence of copulatory organ, location of ovary anterior to testes, totally posterior location of the vitellaria and their quite different and compacted form. Only after an extended study of the difficult problem of distome relationships would it be possible to form any conclusions as to the relationships of this form. I have accordingly left the discussion of the problem of taxonomic position for a later paper.

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ABBREVIATIONS

<i>at</i> , atrium or meeting place of the male and female ducts.	<i>pph</i> , prepharynx
<i>cy</i> , cyst wall	<i>ph</i> , pharynx.
<i>dej</i> , ejaculatory duct.	<i>pr</i> , prostate gland cells.
<i>exa</i> , anterior excretory cavity	<i>sps</i> , seminal vesicle.
<i>exp</i> , posterior excretory cavity.	<i>sh</i> , edge of sheath of ventral suckers.
<i>int</i> , intestine.	<i>shg</i> , shell gland.
<i>lc</i> , canal of Laurer	<i>spl</i> , left spermary.
<i>mi</i> , sphincter muscles	<i>spr</i> , right spermary
<i>nr</i> , nerve collar	<i>uta</i> , ascending uterus
<i>oc</i> , eye	<i>utd</i> , descending uterus
<i>od</i> , oviduct.	<i>vsp</i> , posterior ventral sucker.
<i>oe</i> , oesophagus	<i>vt</i> , vitellaria.
<i>os</i> , oral sucker.	<i>ytd</i> , yolk duct.
<i>ov</i> , ovary	<i>ytr</i> , yolk receptacle.

All drawings unless otherwise stated are from outline camera lucida drawings, the Zeiss-Abbe camera being used in all cases.

The scale drawn on many of the figures is a camera view of the lines of a stage micrometer of the same magnification as the object, it is either 0.1 mm. for low power or 0.01 mm. for high power views.

A STUDY OF SOME EPITHELIOID MEMBRANES IN MONAXONID SPONGES

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TWENTY-ONE FIGURES

In the course of some experiments dealing with the regenerative power of the tissues in certain monaxonid sponges, it became necessary to learn the histological peculiarities of the epidermis in these forms. It soon developed that the adult epidermis in these species did not conform to the type usually thought of as well nigh universal in sponges. A study of the regeneration of the epidermis in cuttings was then undertaken, rather with the idea of its throwing light on the adult structure. During the study of the epidermis some new facts as to the way in which pores close were made out. Finally for the purpose of comparison with the epidermis, the canal epithelium in a suitable species was studied.¹

THE EPIDERMIS IN STYLOTELLA

The most abundant sponge in Beaufort harbor is *Stylotella heliophila*, a form which I have described in a paper now in press for the U. S. Bureau of Fisheries. The genus falls in the hali-chondrine monaxonida. The sponge has well marked ascending lobes of conical shape which bear terminal oscula. The pores are scattered over the whole surface. Spaces of considerable size (subdermal cavities) belonging to the afferent system lie

¹ The work was carried on during the summer of 1909 at the Beaufort Laboratory of the U. S. Bureau of Fisheries. My thanks are due to Hon. Geo. M. Bowers, U. S. Commissioner of Fisheries, for a place in the laboratory, and to the Director, Mr. H. D. Aller, for his kindly aid during my stay

close to the surface, and as is customary in such sponges imperfectly separate a thin superficial layer known as the dermal membrane from the inner mass of the sponge body. The dermal membrane contains no flagellated chambers, or only a very few scattered here and there, and is made up of a thin sheet of mesenchyme containing spicules, which is covered on the outer surface by the epidermis and on the inner surface by the epithelioid membrane forming the wall of the subdermal space (and of the canals in general). According to the current conceptions of the histological structure of sponges we would expect to find the epidermis and canal walls both to consist of a single layer of flat epithelium cells (pinacocytes).

Actually I find that the epidermis of this sponge consists of a thin protoplasmic sheet studded with nuclei and exhibiting absolutely no cell boundaries. It is a syncytium. Cell boundaries are sometimes overlooked but it seems to me that the variety of histological methods I have practiced makes it certain that cells do not exist.

Results with material fixed in alcohol

Comparison with living tissues shows that strong alcohol, absolute or 95 per cent, is an excellent fixative for sponge tissues. I use it in liberal quantities, and very shortly after the immersion of the piece of sponge, change to fresh alcohol, changing again after a few hours. The precipitate which alcohol unfortunately causes in sea water has scarcely time to settle on the sponge if the first change be made quickly. Moving the piece about in the alcohol also helps to keep the surfaces clear of the precipitate. Suitable pieces were stained in toto with haemalum and were then imbedded, some in celloidin, some in paraffine. On the whole I recommend the celloidin, but the paraffine preparations were satisfactory. After the xylol bath I add soft (40° melting point) paraffine to the xylol, warm the mixture up gradually, and imbed 30 minutes in soft and 30 minutes in harder (50°-55° melting point) paraffine. Very thick tangential sections are made. Such

sections are far better than thin ones. They afford many places where, owing to the transparency of the tissues underlying the epidermis, the latter can be well seen. The sections were given an after stain with congo red, or with Delafield's haematoxylin followed by congo, and were mounted in balsam. To obviate the possible ill effects of imbedding, strips of the epidermis were torn off with forceps from the alcoholic material, were stained in haemalum and congo red, and mounted in balsam. Most of the pieces obtained in this way are too thick for study, but occasionally very thin strips peel off.

As is well known the pores of sponges close and open. Preparations of the epidermis with the pores widely open were made from sponges that had been kept in a live box. In a live box placed where the tidal current is good, the sponge is usually found with the oscula and pores fully open and the canals dilated. If the sponge so expanded be suddenly plunged in the fixative, the pores will not have time to contract. More useful preparations are those in which the pores are closed or half-closed. Sponges that have been kept a short time in running aquaria are found to be in this condition.

A part of the dermal membrane as seen in a thick tangential section is shown in fig. 1. Some of the pores are completely open and others nearly so. The wall of a small subdermal cavity is indicated by the line *s.c.w.*, and into this cavity the pores open. Beyond *s.c.w.* we come to a thicker part of the body separating the subdermal space shown from neighboring ones. In this part a few especially conspicuous mesenchymic cells, *m.c.*, appear. They come into view when the microscope is focussed just below the surface of the sponge. The canal wall shows some of the lining cells, *c.c.*, as seen in optical section. They also appear of course only at a focus below the surface of the sponge. Over the subdermal cavity the figure shows the epidermis in focus. It appears as a continuous thin protoplasmic sheet without cell boundaries and studded with nuclei, *ep.n.* On focussing below the epidermis the mesenchyme cells of the dermal membrane would come into view. Below the mesenchyme lies the inner covering of the dermal membrane, an epithelioid layer contin-

uous with the canal lining in general. Round most of the epidermal nuclei the protoplasm is aggregated, forming thickened more deeply staining areas which shade off into the internuclear portion. The structures marked *p.m.*, which I propose to call pore membranes, and which so far as I know have not been described, are extensions of the epidermal sheet over the pores. These extensions are so thin that they afford an especially favorable opportunity for studying the intimate structure of the epidermal layer. Their nature is learned when the process of pore closure is studied, and it will be well now to give a description of this process.

The dermal pores of the monaxonid sponges are customarily referred to as mere perforations of the dermal membrane. They are in reality short canals leading from the exterior into the subdermal chambers. Ordinarily the dermal membrane, while thin, is of such thickness that the actual aperture at the surface of the sponge is distinguishable, on focussing, from the canal itself. Thus in fig. 1, the pore membrane, *p.m.*, partially closes the aperture and is distinctly seen when the epidermis is in focus. On focussing a little lower the wall of the canal itself, *p.c.*, comes into view as a distinct line which often exhibits a nucleated thickening or two. The nucleated thickening may as in the case of two of the pores shown in fig. 1, extend out into the mesenchyme in the shape of a slender process. I propose to restrict the use of the term pore (*viz.*, dermal pore), to the actual aperture, and to designate the short canal as the pore canal.

When the pores are widely open as is the case with pore 1, in fig. 1, there is no sign of a pore membrane. The epidermis is directly continuous at the edge of the pore with the lining of the pore canal. But even when the sponges are fixed at once on being taken from the live box, some of the pores will be partially closed and will show the pore membrane, *p.m.* This thin extension of the epidermal sheet in sponges so preserved will usually be found barely extending beyond the margin of the pore and it may or may not include a nucleus. It is a single thin layer which yet is continuous with both epidermis and the lining of the pore canal. Since it has the structure of the epidermis I speak of it and regard

it as an extension of that layer. If the sponge has been kept in an aquarium a short time, preparations show that the pores are for the most part about half closed. In fig. 3 two such pores are shown as they appear in a thick tangential section similar to that from which fig. 1 was made. The pore membrane, *p.m.*, here extends well over the pore canal. If the sponges have been kept some time in the aquarium, regions will be found in which the pores are closed. Fig. 2 represents the dermal surface of a thick tangential section. The region shown lies over a subdermal cavity and the pores are closed. The outlines of the pore canals, *p.c.*, are visible on focussing just below the surface. The thin sheet, *p.m.*, covering in the pore canal is the pore membrane. A comparison of such preparations shows plainly that the pores are closed by a thin extension of the epidermis over the pore canal.

Owing to the peculiarities of the species, especially unevenness of surface, abundance of spicules, and abundance of amoebocytes, it is well nigh impossible to observe the closure of the pores in living preparations of the dermal membrane as made from the normal sponge. Free hand tangential sections of the living sponge were sliced off, but these proved of no value. Pieces were cut out from the upper part of the oscular lobes in very transparent regions and where the wall of the lobe is thin, but these again were useless for the purpose. I did succeed, however, in observing the closure of the pores in life by practicing the following method.

Free hand sections about one-eighth inch thick were made transversely through an oscular lobe, and therefore directly across two or three of the main efferent canals. Such a section when cut is a circular piece of sponge tissue perforated by the segments of these canals. The segments of the canals are of course open above and below at each surface of the section. If such a section be kept a day in an aquarium a new dermal membrane develops over both surfaces and over the open ends of the canals. The new membrane closing in the canals is smooth and contains but few spicules. If the section now be mounted in sea water under a cover glass the new membrane over the canals may be studied with a high objective. It will be found to contain pores

and one may actually see that these are closed through the creeping of the most superficial layer of the dermal membrane (newly formed epidermis) across the pore, *i.e.*, over the aperture of the pore canal, thus giving rise to a pore membrane.² As to the opening of the pores after closure by the pore membranes, I have no actual observations, but it is obvious that the pore must reappear as a perforation in the membrane, which then recedes towards the margin of the pore canal.

The question may be asked, is the pore canal a permanent structure, or does it too close up? Since the pore canal perforates the dermal membrane its closure obviously could only be brought about through an extension of the mesenchyme of that membrane. In *Stylotella* I always find the pore canals distinct even when the pores are completely closed. Hence the pore canals must be regarded as structures that are permanent in ordinary conditions of the sponge. My observations on *Reniera* and *Lissodendoryx* (*vide infra*) nevertheless show that the pore canal itself may be partially or completely obliterated in monaxonid sponges.

Another question may be asked before we leave this matter of the pore and its closure. Is there any one nucleus that is especially associated with a pore membrane? An examination of figs. 1, 2 and 3 shows there is no such nucleus. The pore membrane may spread to a considerable distance over the pore canal before any of the epidermal nuclei enter it (fig. 3), and when the pore is completely closed (fig. 2) the membrane may show a nucleus somewhere near its centre or again one or two nuclei near or at its margin. The epidermal nuclei are irregularly distributed, in some spots close together, in others farther apart. This is well shown in fig. 2. These nuclei moreover are all alike. The facts would seem to indicate that the epidermal sheet of protoplasm spreads of its own initiative over the pore canal, and that the nearest nucleus or nuclei are simply drawn into it. In *Reniera* on the other hand there is always one nucleus at the margin

² In the healing up of such a section a considerable rearrangement of the canal system certainly takes place. A new osculum is established, and this apparently may develop at any point on the surface of the piece. Pore canals lead through the newly formed dermal membrane into what were originally main efferent canals.

of the pore, and this nucleus is (perhaps only passively) associated with the formation of the pore membrane.

Some details in the structure of the epidermal layer remain to be mentioned. The irregular distribution of the nuclei has been noted. They are small and uniformly exhibit only a nuclear membrane and a few chromatin granules in the nucleoplasm. No cases of division were observed, although mitotic figures in amoebocytes of the mesenchyme were noticed not infrequently. Round each nucleus or group of two or three is a more deeply staining area which appears finely granular or granular and reticular. The rest of the membrane stretching between the nuclei and over the pore canals exhibits a fine reticular structure. The reticular structure is found everywhere, but is most distinct in the thin pore membranes. Discrete granules are absent or nearly absent in the epidermal sheet. It should be understood that the reticular appearance of the epidermal layer is perhaps only the optical expression of an alveolar structure. To demonstrate the reticular appearance a good immersion objective is necessary. I have chiefly used Zeiss 2 mm. ap. 1.30 but also Zeiss 2 mm. ap. 1.40, with comp. oculars 6 and 8. Very white clouds on sunny days afford satisfactory light.

Results with other fixatives

Material fixed by other methods confirms the account just given.

Picro-sulphuric. Tangential sections and strips of epidermis were prepared from material fixed in picro-sulphuric. The staining was as for the alcoholic material, and the preparations gave the same results. Absolutely no cell boundaries exist. The internuclear sheet more commonly appears finely granular rather than reticular. Possibly this is due to a deeper staining of the nodal points. But the fine reticular structure comes out well in places, especially in the pore membranes. Discrete granules such as are found in mesenchyme cells are either entirely absent or are found only in very small number here and there.

Acetic acid. Pieces were fixed in glacial acetic for a few minutes (5-10) and then transferred to water. The dermal membrane was peeled and cut from the choanosome, and was then cleaned of the underlying sponge parenchyma which was picked away with forceps and needles. The pieces were then stained, some in methyl green, others in acetic carmine or in haemalum, and were mounted in glycerine. Preparations so made give results similar to the foregoing. But they are not as transparent as balsam preparations and do not disclose the detailed structure of the internuclear sheet.

Osmic acid. Pieces were fixed in one-half per cent osmic for 10-15 minutes, washed in running water, and hardened in Müller's fluid 12 hours. They were run up very gradually through the alcohols. Sections and strips were made, stained in haemalum, and mounted in balsam. The preparations very frequently exhibited interesting artefacts. At first sight an epithelium seemed to be marked out in the clearest way. Perfectly clear channels of considerable width cut up the surface layer into areas that were often polygonal. Examination with an immersion objective showed that these areas were not cells. They sometimes have nuclei and sometimes not, and the channels between the areas exhibit peculiarities in their course which clearly indicate them to be cracks. The whole appearance must be due to the cracking of the very delicate epidermal sheet. The fixative probably makes the sheet brittle, and it later cracks perhaps during the washing.

Sublimate. Pieces were fixed for a few minutes in saturated corrosive sublimate and washed in iodised 70 per cent alcohol in the usual way. Tangential sections and strips of epidermis were prepared and stained in haemalum and congo red. Such preparations frequently exhibit artefacts similar to those produced by osmic. The surface layer is broken up into thin and irregularly polygonal pieces that are widely separated by perfectly clear channels. The latter are crossed in some places by a few slender protoplasmic filaments. Careful examination shows that the pieces are certainly not cells. Some are without nuclei, others with a nucleus or sometimes with two. They often include one

or more large clear vacuole-like spaces. This appearance again is probably due to cracking of the epidermal layer, perhaps coupled with a violent coagulation set up by the sublimate. The appearance is certainly an artefact, although the pieces in many places look at first sight like cells.

Silver nitrate. Thin pieces were sliced off from the surface of a living sponge, and were fixed 5-10 minutes in one-twentieth per cent osmic acid. After thorough washing in distilled water, they were transferred to one per cent silver nitrate and exposed to direct sunlight 5-10 minutes (Hertwig's method). After washing and running up through the alcohols, strips of epidermis were peeled off and mounted in balsam. Tangential sections were also made and mounted in balsam. As a control small hydromedusae were stained in the same way. The subumbrellar surface of the latter showed the usual polygonal network of distinct brown lines, marking out the epithelium cells. The method was employed several times on favorable days.

Stained in this way the surface of *Stylotella* frequently exhibits no lines that in any way suggest cell boundaries. But in places an appearance is got with a Zeiss D objective as if epithelium cells were marked out. Examination with an immersion objective shows that the appearance (fig. 4) is due to artefacts and not to the presence of epithelium cells. The facts may be summed up as follows. The network of lines is below the thin surface layer. The lines are no browner than other strands, viz., have the osmic and not the silver stain. In the meshes are irregular masses that are usually nucleated. The areas marked out by the lines may vary greatly in size. It is plain that such areas cannot be epithelium cells. The appearance is probably caused by violent coagulation of mesenchyme cells and strands. Inter-cellular connectives and parts of cell bodies remain as the network of brown strands, while the cell bodies, contracted and torn loose from the connectives, remain as the irregular masses that lie in the meshes.

REGENERATION OF THE EPIDERMIS IN STYLOTELLA

A dermal membrane with normal epidermis soon regenerates over a cut surface. For the study of the process of regeneration, sections vertical to the surface are of little use. The method I have followed was to allow the regeneration to proceed a certain time, then to fix and harden the piece of sponge, and to cut from the superficial region a number of thick (100μ) tangential sections. For the fixation alcohol, picro-sulphuric and sublimate were employed. The piece was stained in toto with haemalum, and the sections with congo red. Paraffine and celloidin sections were chiefly used, but good preparations were sometimes made by slicing off free-hand the regenerating surface from the piece in alcohol, and at once staining and mounting the slices. Or the piece was fixed in glacial acetic, washed in water, and the regenerating surface sliced off. The sponge parenchyma was then picked away with needles and forceps from the surface layer, which was later stained and mounted in glycerine.

The original cut surface was made as smooth as possible, and all of it is included in the first few sections. These are mounted with the regenerating surface uppermost. Where the surface was part of the choanosome such preparations are too opaque for study. But where the surface was part of the transparent collenchyma, the sections offer fairly clear pictures. Much the best pictures of all are to be had from the new dermal membrane which develops across the cut ends of the larger canals. To obtain membrane of this kind I cut off oscular lobes about an inch below the apex, thus cutting the main efferent canal transversely. The open ends of the canals become closed in by the new membrane which extends out from the surrounding collenchyma across the aperture. The rate at which the canals become closed in may be gathered from the following record. The lobes were cut off at 9 30 a.m., the cut surface of each lobe showing several widely open canals. At 1.30 p.m., most of the canals were closed in by thin, collenchymatous membranes perforated in the centre like diaphragms. In the case of a few canals the membranes had completely closed the apertures. Within an hour or two all of

the membranes had completely formed and the canals were entirely closed in. In fig. 5 one of the newly formed membranes, *c.m.*, with surrounding collenchyma and outlying choanosome is shown.

When the cut is first made, the dermal membrane covering the rest of the sponge ends at the exposed surface with a sharp edge. On the cut surface itself are exposed in choanosomal regions, flagellated chambers, mesenchyme, and spicules; in the regions immediately round the larger canals, only collenchymatous mesenchyme. The mesenchyme everywhere includes branched cells freely interconnected, and free amoebocytes. The latter are scarce in the collenchyma. Collenchymatous mesenchyme is especially characterised, it will be remembered, by the large amount of watery intercellular substance and the considerable length of the cell processes. A recognisable new dermal membrane develops over the whole surface within a day. The edge of the old membrane remains distinguishable for some hours, but it applies itself closely to the more solid sponge tissue, sinking in to meet the latter where it had covered subdermal spaces, and after about 12 hours it is no longer recognisable. By this time it is in perfect continuity with the layers of mesenchyme cells stretching over the cut surface and which are developing into the new dermal membrane.

We may now proceed to the detailed examination, by stages, of the developing dermal membrane and epidermis, using for study as explained above the membranes that develop across the open ends of canals and over collenchymatous regions.

One hour after cutting. The cut surface is occupied by branched cells containing abundant and conspicuous granules. Even in a comparatively small area they exhibit slight differences of level. These cells are interconnected so as to form a fairly close network. Some very small spheroidal cells, probably metamorphosed collar cells, lie free here and there. Many of the superficial granular cells are thin and flattened. Below the superficial cells lie several layers of essentially similar granular cells which are not flattened. They are interconnected with one another and with the superficial cells. The entire network formed by the granular mesenchymal cells is closest at the cut surface and becomes more and more open as we go deeper below the surface.

Two hours after cutting. The cells at the surface are now more uniformly flattened than they were an hour earlier. A group of the superficial cells is shown in fig. 6.

Five hours after cutting. The surface is now occupied by a layer of thin, flattened, coarsely granular cells or cell areas connected by a complex network of fine intercellular strands (fig. 7). The cells areas are mostly uninucleate but may include two or even three nuclei. The areas have no precise boundaries but merge gradually into the intercellular network. On focussing below the surface layer, coarsely granular mesenchymal cells come into view. These have slender processes and are freely interconnected forming a coarse open network (fig. 8, *m.c.*). This open network of coarsely granular mesenchymal cells constitutes the body of the developing dermal membrane. In its spaces which doubtless represent pore canals have already appeared. One such is shown in fig. 8 (*p.c.*). The mesenchyme cells bounding it, and which doubtless become the lining epithelium, do not yet form a continuous wall. Above the developing pore canal the epidermal layer, *p.m.*, is shown as it appears at the upper focus.

Twelve hours after cutting. The surface is now occupied by a continuous epidermal membrane in which the cells that have fused are still distinguishable (fig. 9). The area round each nucleus or group of two or three takes a deeper stain and appears as a finely granular, vaguely delimited area containing a good many of the coarse granules that characterise the fusing cells in earlier stages. Between these areas the epidermal membrane is a thin continuous sheet which in places appears reticular (alveolar) and in other places more fibrillar. In this thin sheet one sees here and there a few of the coarse granules which seem to be lodged, in gases at least, at the nodes of the reticulum. The sheet exhibits small perforations of varying size, sometimes twice as large as that shown in fig. 9 (*per.*). Possibly these are the beginnings of pores, although I was not able to observe that they always lay over pore canals. In a regenerating dermal membrane at this stage groups of well formed pore canals are found here and there (fig. 9, *p.c.*). In the preparation shown in fig. 9, the pores are open.

Later development. The epidermis 24 hours after cutting is like that of the normal sponge. The coarse granules found in earlier stages are absent or present only in scanty number here and there. Pore canals that are open or closed in by pore membranes are abundantly present. The ectosomal skeleton is scanty. Pieces of sponge were kept in live boxes for a week and in these examination indicated that the ectosomal skeleton was practically like that of the normal sponge. The color of the new surface at this time was still like that of the interior, orange, while the old surface was orange with a distinct tinge of green.

Summary. A comparison of the stages just described shows that immediately after the cutting coarsely granular mesenchyme cells approach the exposed surface in considerable number. Many of the migrating cells are doubtless originally free amoebocytes. The granular cells when they have reached the neighborhood of the surface appear as branched bodies freely interconnected. This layer of interconnected granular cells develops into the new dermal membrane. The cells at the surface become flattened and more closely set than the deeper elements from which they are no doubt recruited during the first few hours. They fuse to form the epidermis. Union between the cells takes place not through crowding so as to give rise to plane surfaces, but through the continued development of intercellular connectives. As these become more numerous and branched they give rise to a complex reticulum of protoplasmic strands. This intercellular reticulum becomes transformed into what we would usually speak of as a continuous sheet of protoplasm, although careful examination shows that even in the adult it has a finely reticular, possibly alveolar, structure. During the metamorphosis of the superficial granular cells into the epidermis, the cells lose their characteristic granules. The pore canals arise as excavations in the mesenchyme of the developing dermal membrane, and are covered in by the new epidermis which in such places constitutes pore membranes.

THE EPIDERMIS IN RENIERA

The species used is an undescribed one fairly common in Beaufort harbor. The body, frequently about 100 mm. high, is a complex system of anastomosing cylindrical branches, the diameter of which varies from about 3 mm. to 8 mm. The color is often pink but varies to a brown. The oscula terminate short tubes arising vertically from the branches. Such oscular tubes are frequently 1.5–3 mm. in diameter, 2–4 mm. high. The wall of the tube is colorless, thin, and transparent. The sponge, like the other two forms used for the observations recorded in this paper, falls in the halichondrine monaxonida.

For the study of the epidermis pieces were fixed in absolute alcohol, 95 per cent alcohol, sublimate, picro-sulphuric. Thick tangential sections were made from the smoothest and most transparent parts of the surface. Both celloidin and paraffine were employed. Useful preparations were also made directly from the oscular tubes in the following way. The tube was cut off, split lengthwise, the sponge tissue picked away from the canalar surface, and the pieces mounted with the epidermal surface uppermost. For staining I made use in general of haemalum and congo red, staining the piece in toto with haemalum and the sections in congo.

Results with material fixed in alcohol

Alcohol proved much the best fixative. The epidermis is so delicate a membrane that during the treatment necessary with other fixatives it cracks. In the alcoholic preparations clean places must be looked for. These are abundant enough, and in such places the structure of the layer may be successfully studied. There are no cell boundaries. The layer is a syncytium as in *Stylotella*, consisting of a thin, continuous sheet of protoplasm containing abundant nuclei that are irregularly scattered (fig. 11). Round each nucleus as a rule the protoplasmic sheet is thicker than elsewhere, takes a deeper stain, and presents a finely granular appearance. The rest of the sheet is minutely reticular.

Granules sufficiently large to be recognised individually and which are abundantly present in mesenchyme cells, are not found in the epidermis. The reticular character of the sheet is very distinct in places where the staining is both deep and clean. The meshes appear to be actual spaces. They look clear and empty and are bounded by the stained reticular lines. The thin pore membranes closing in pore canals are especially favorable for such observations. The epidermal sheet is certainly of surprising delicacy and thinness. It may often be traced over the large spicules which lie horizontally and form the superficial meshes of the skeletal network. In such places it rests upon the white background of the spicule and the reticular character comes out distinctly. There are places where mesenchyme cells of the dermal membrane also lie on top of the superficial spicules. But it is where the epidermis alone crosses the spicule that the opportunity for study is so especially good.

Results with other methods

Sublimate was given a good trial as a fixative. The epidermis cracks a great deal. The fragments are sometimes fit for study. They exhibit the reticular character of the sheet and an absence of cell boundaries, as noted above.

Picro-sulphuric which is a good fixative for the epidermis in *Stylotella* does not give good results on *Reniera*. The epidermis cracks into pieces. When treated with this fluid the membrane seems to have no stiffness. Thus it often drops down into the pore canals and breaks away from the part left on the surface. The fragments are sometimes fit for study. They are frequently polynucleate but exhibit no cell boundaries. The reticular character of the sheet could not be observed on this material.

Several trials of the silver nitrate method were made on favorable days. The silver entirely failed to show the presence of cell boundaries in the epidermis. Where the stain is deep, the outlines of mesenchyme cells and processes sometimes appear. The silver was used according to the method already described for *Stylotella*. Oscular tubes that had been so stained were

split and mounted in water, glycerine, and balsam. Tangential sections were also made. As a control pieces of an expanded *Leptogorgia* were used, and the epithelial cells on the surface of the polyps were here outlined with great distinctness.

Pores, pore canals, and pore membranes

In the preparations made from preserved material, the pores are sometimes wide open or partially, sometimes completely, closed by pore membranes. The pore membrane as in *Stylotella* is simply an extension of the epidermis. When it incompletely closes the pore it has a single nucleus (fig. 11,), and even when it is complete it may have but one (fig. 11,). Frequently, however, when it is complete it exhibits more than one nucleus (fig. 11, b). As long as the pore membrane is imperfect the outline of the pore canal is distinct. When the pores are completely closed however it often happens that the outline of the pore canal is vague or lacking at some part of the circumference (fig. 11). The explanation of this appearance must be that after the epidermis has extended over the pore canal the mesenchyme of the dermal membrane also extends in towards the middle of the canal, thus tending to obliterate it.

Fortunately in this sponge the behavior of the pores may easily be watched during life. For this purpose an oscular tube is cut off, split lengthwise, and the halves mounted with epidermal surface uppermost in plenty of sea water under a coverglass. The cover flattens the pieces sufficiently to permit the use of a one-sixth inch objective. In such preparations made from a sponge just removed from the live box, many of the pores will be found open and their closure may be actually observed. I append the following records of observations on the closure of selected pores.

Pore 1. At 9.45 a.m. the pore is open with one nucleus at the margin (fig. 10). The nucleus shifts its position traveling back and forth along the margin, going sometimes half round the pore and back again. The movements of the nucleus are quick and easily observed. At 9.50 the epidermis extends a short distance

over the margin in the shape of a thin film. This gradually spreads across the pore becoming a well marked pore membrane. As it spreads the originally marginal nucleus passes into it. The sketches (fig. 10) show successive stages in the passage of the membrane across the pore. At 9.58 the pore canal is almost completely closed in. Its outline is still distinct at this time. Five minutes later the pore is completely closed, and the outline of the pore canal is no longer distinguishable.

Pore 2. The pore at 10 a.m. is partly closed by a few interconnected strands of protoplasm which include a nucleus (fig. 12). The strands are thin and delicate, and are in continuity with the surrounding epidermis. The protoplasmic strands change form and arrangement, and the nucleus shifts its position, all very quickly. Such amoeboid movements continue for some minutes. During their progress camera sketches were made, and the conditions at 10.05 and 10.07 are shown in fig. 12. By 10.10 the protoplasmic strands have taken the shape of a marginal film. This is drawn into the epidermis, the nucleus remaining at the margin of the pore, and at 10.12 there is only the usual appearance of an open pore. The nucleus now shifts quickly back and forth along the margin of the pore, narrow marginal films appearing and disappearing as the nucleus changes position (comp. sketches drawn at 10.15, 10.17, 10.20, fig. 12). The narrow marginal film present at 10.20 begins to spread at 10.21 and rapidly covers the whole pore, becoming a pore membrane into which the nucleus passes. Two stages in the completion of the pore membrane are drawn as they appear at 10.23 and 10.25. The pore is completely closed by 10.27. The wall of the pore canal was distinct all round until 10.21. Shortly after that time it began to grow indistinct round a part of the circumference (right side). At 10.25 it was no longer distinguishable in this region and was only vaguely outlined on the opposite side. The pore canal was kept under observation until 10.40 a. m. At that time its outline (*p.c.* in fig. 13) was still vaguely distinguishable, although circumscribing a much smaller area than formerly.

Pore 3. When the observations began the pore canal (fig. 14, 1.45 p. m.) was far smaller than the normal. It had evidently

already contracted. It was partly covered by a pore membrane at the margin of which lay a nucleus. The marginal pore membrane was then largely drawn into the epidermis, the nucleus shifting its position in what remained (comp. sketches drawn at 1.50 and 1.53). At 1.53 the marginal membrane began to spread rapidly, closing in the pore by 1.57. After complete closure of the pore the outline of the pore canal was still distinguishable. The outline was distinguishable but smaller at 2 p.m. The wall at this time was far from sharp and appeared rough and granular, whereas before closure of the pore it was sharp and smooth. The rough outline at 2 p. m. probably indicates how far the mesenchymal jelly has spread towards the middle of the original pore canal.

Pore 4. The pore at 2.05 p.m. was wide open, and at the margin two nuclei were distinguishable (fig. 15, 2.05 p. m.). One nucleus, *a*, remains at rest, but the other nucleus, *b*, shifts its position back and forth in the usual way. Its position at successive moments is shown in the camera sketches made at 2.07 and 2.10. Nucleus *b* is the pore nucleus, the movements of which are associated with the formation of the pore membrane. The other nucleus *a* is not especially concerned in the closure of the pore. At 2.12 the epidermis has just crept beyond the margin of the pore, carrying with it the nucleus *b*. By 2.15 the pore membrane has completely crossed the pore and the outline of the pore canal is indistinguishable.

Pore 5. At the beginning of the observations (fig. 16, 3.15 p.m.) the pore which is somewhat constricted is crossed by a single strand of protoplasm. The strand moves across the pore and incorporates the nucleus (3.20). The strand with the nucleus at its base now shifts its position across the pore back and forth, finally passing to the edge and becoming a marginal film (3.30). This quickly spreads over the pore in the usual way.

Pseudopodial activity at the pores

While making observations on the closure of pores, pseudopodial activity was occasionally observed at the margin of the pore

and at the free margin of a partial pore membrane. In fig. 17 three pores are represented in which such activity is going on. In pore *b* the pseudopodia extend out from the incomplete pore membrane, in the other two cases from the margin of the pore itself. The fine pseudopodia were thrown out, moved about quickly, often fused more or less with one another, sometimes combining to form a network (pore *c*). They then were partially or completely drawn in, but reappeared after a short interval. This remarkable phenomenon was observed in the case of the three pores shown during one-half hour, at the end of which period the pores were still wide open and the pseudopodial activity going on. At several other times I have noticed the formation of one or two flagellum-like pseudopods at the margin of open pores. Such pseudopods would quickly appear, move or wave from side to side, and be drawn in. There was nothing to indicate that this pseudopodial activity at the margin of pores was a pathological phenomenon. It is possible that it occurs commonly during life and that the pseudopodia are temporary, sensory processes which explore, so to speak, the region of the open aperture. The facts afford a further illustration of the widespread occurrence of "filose phenomena," to the importance of which as an expression of the fundamental nature of protoplasm, Professor and Mrs. E. A. Andrews have repeatedly called attention (see especially, Andrews G. F., '97).

Summary account of pore closure in Reniera

The pore canals may undoubtedly contract, viz., while still open become smaller (comp. figs. 15 and 16). The entire thickness of the dermal membrane shares in this process. Actual closure is however brought about by an extension of the epidermal layer across the pore. This extension of the epidermis may at once constitute a simple and continuous pore membrane (fig. 10) similar to that present in *Stylotella*. Or the epidermis may first extend across the pore in the shape of one or more strands of protoplasm which shift about in amoeboid fashion (figs. 12 and 16) and are then withdrawn into the general layer before the continu-

ous pore membrane finally begins to extend across the pore. A single nucleus not differing in appearance from other epidermal nuclei is associated with the closure of a pore. It lies at the margin round which it is shifted back and forth, in the first stage of closure, probably by wave-like movements of the protoplasm similar in some respects to those occurring in plants cells (*Nitella*, *e.g.*). These movements of the nucleus are quick and easily observed. For instance a nucleus made the complete circuit of a widely open pore in about one minute. After closure of the pore, other nuclei beside the pore nucleus may pass into the area which covers the pore canal (fig. 11). The constant presence of a nucleus at the pore and its quick changes of position strongly suggest that it is in some way physiologically concerned in pore closure.

The extension of the epidermis to form a pore membrane does not necessarily involve the rest of the dermal membrane, and hence after the epidermis has spread across the pore, the pore canal may still remain open, in which case its outline is distinguishable on focussing below the surface. Usually after closure of the pore, the outline of the canal is suddenly lost to view. This must be due to centripetal streaming of the mesenchyme of the dermal membrane, induced by some local contraction in the epithelial wall of the canal. Perhaps in nature the pores commonly remain in this condition until they reopen. At any rate this is the state in which closed pores are usually found in preserved material (fig. 11). In excised pieces of sponge kept under a cover glass the pore canal may completely or almost completely disappear. In this latter case the area of the canal diminishes greatly in size and its outline becomes rough and vague (figs. 13 and 14). This small and vaguely outlined area represents the central region of a pore membrane, and indicates how far the mesenchyme of the dermal membrane has streamed inwards in its obliteration of the pore canal.

The formation of a pore membrane is a contraction phenomenon which involves only the epidermis. The closure of the pore canal is probably also primarily a contraction phenomenon, which in this case involves the epithelial lining of the pore canal. The epithe-

lial lining contracts, we may suppose, after the fashion of a sphincter, locally or throughout the extent of the pore canal, and so tends to obliterate the lumen. Such contraction brings with it a centripetal streaming of the mesenchyme of the dermal membrane. The closure of the canal is certainly not brought about by the contraction of surrounding fibre-like cells arranged in sphincter fashion. There are none of these.

In the centripetal streaming of the dermal mesenchyme we must distinguish active movements of cells and passive movements of intercellular jelly. Both undoubtedly occur. On the active movements of such cells I may record the following few observations. The conspicuous cells in the mesenchyme of the dermal membrane are coarsely granular amoebocytes (cells *a*, *c*, in fig. 13) and pale cells either without coarse granules or with only a few (cells *d*, *b*, in fig. 13). Both varieties of cells may appear in the spheroidal shape. When they are actively moving they are irregular in shape, the body extending out into slender prolongations. Cells of both varieties constantly shift their position, and undergo changes of form, all very slowly. The granular amoebocytes move more actively than the pale cells. The cells *a*, and *c* crossed the space included between the spicules, passing over *b* which they obscured for a time. In crossing the space, cell *a* consumed five minutes.

CLOSURE OF PORES IN LISSODENDORYX

The species used was *Lissodendoryx carolinensis*, a common form in Beaufort harbor, and a description of which is contained in a paper now in press for the U. S. Bureau of Fisheries. The sponge falls in the halichondrine monaxonida. The whole surface is abundantly covered with tubular translucent papillæ the walls of which are perforated with numerous pores. These pore-papillæ which are often slightly branched are contractile and may almost entirely disappear. When dilated they are about 3-5 mm. long and 1 mm. in diameter.

If such pore-papillæ in the expanded state are cut off and mounted in sea water, many pores are found to be open, and their

closure may be watched under the microscope. Each pore lies in a field surrounded by long spicules (tylotes) and when expanded is large. As in the cases of the preceding species, I restrict the term pore to the actual aperture at the surface, using the term pore canal for the very short tube which perforates the wall of the pore-papilla. I append the following record of observations on the closure of selected pores.

Pore 1. When the observations began at 2.25 p.m., the pore canal and pore were wide open. The pore canal steadily contracts until 3.20 p.m. The diameter of the canal at this time is about one-third of the original size. While the pore canal is contracting, the surrounding spicules come closer together (the whole papilla contracts). The mesenchyme cells at 3.20 extend to the very wall of the pore canal. Immediately after 3.20, a thin homogeneous looking membrane containing no mesenchyme elements suddenly extends out across the open aperture. This pore membrane (fig. 18, 3.25 p. m.) in a few minutes time closes the pore completely.

Pore 2. The pore canal at 3.30 p.m. had already contracted to about one-half the full size, and was in the same condition as pore 1 at 3.20. The pore, however, closes in a different way from pore 1. The canal steadily contracts until it disappears, at 3.40 p.m.

Pore 3. The pore canal contracts to about one-half its original diameter. It then is found covered in near its margin by an extension of the dermal membrane. This extension constitutes zone *b* of fig. 19. From this zone a further extension in the shape of a very thin membrane, zone *a*, formed exclusively by the epidermis, extends over the more central part of the canal. It seems proper to designate zone *a* as a pore membrane. My record for this pore is not complete. Probably the pore membrane was first formed, and the mesenchyme of the dermal membrane later streamed inwards, forming zone *b*. The pore membrane soon closes the pore completely. The distinction between zones *b* and *a* is later lost, since granular amoebocytes and microscleres invade zone *a*. Even after this has occurred, on focussing below the surface, the wall of the pore canal may be seen.

Summary. In this sponge the pores do not always close in the same way. (1) Often the whole pore canal closes up and disappears by simple contraction. Pore 2 closes in this way. (2) In other cases the pore canal shrinks as before, but actual closure is brought about by a rapid extension of the epidermal layer across the pore, forming a pore membrane (pore 1). (3) In still other cases the pore canal shrinks and closure is then effected through the formation of a pore membrane which is gradually reinforced by the dermal mesenchyme (pore 3).

COMPARISON OF THE METHODS OF PORE CLOSURE AS OBSERVED IN STYLOTELLA, RENIERA AND LISSODENDORYX

The various ways in which pores were observed to close in the three species of sponges that were studied may be arranged in a series expressing the successive physiological states that conceivably may occur in the closure of a dermal pore in monaxonid sponges generally. (1) A partial closure of the pore may be brought about by the extension of the epidermis across the aperture in the shape of one or more amoeboid strands of protoplasm (figs. 12 earlier stages, and 16, for *Reniera*). Possibly this state is sometimes preceded by the formation of fine pseudopodia at the margin of the pore (fig. 17, for *Reniera*). Such closure is temporary. The pore opens and then remains open or (2) is completely closed by a continuous extension of the epidermis across it, forming a pore membrane (fig. 12 later stages, for *Reniera*; figs. 2 and 3 for *Stylotella*). (3) To bring about a more secure closure of the pore, the pore membrane is reinforced by a centripetal extension of the dermal mesenchyme induced through contraction of the epithelial lining of the pore canal. This reinforcement extends gradually from the margin across the whole pore (fig. 19 and pore 3, for *Lissodendoryx*). (4) Hitherto the lower part of the pore canal, opening into the subdermal chamber, has remained open. Contraction accompanied by centripetal streaming of the dermal mesenchyme now affects this part of the canal, and almost obliterates it (fig. 13, for *Reniera*) or completely obliterates it (pores 1 and 4, for *Reniera*).

The final result, complete closure and obliteration of the pore canal, which may occur as the end of a series of easily distinguished steps, is in other cases brought about simply through continued shrinking (pore 2, for *Lissodendoryx*). Or the pore canal may shrink greatly, and then be closed in by the formation of a pore membrane (fig. 18, for *Lissodendoryx*), the complete obliteration occurring later.

THE CANAL EPITHELIUM IN *STYLOTELLA*

Oscular lobes of sponges in which the canals were well expanded, were fixed in alcohol (absolute and 95 per cent), sublimate, and picro-sulphuric. After hardening pieces were excised which included two or three of the main efferent canals, and these were sectioned so as to cut the canals longitudinally. Celloidin was used as an imbedding material, and the sections were cut thick. As the series of sections passes through a canal, the first and last sections will of course cut the canal wall tangentially, and these sections when mounted with the canal face up give excellent surface views of the lining. For staining haemalum was used "in toto," and the sections were stained in congo red. It is only the main efferent canals that I have studied.

Pieces fixed in alcohol and picro-sulphuric yield essentially the same results. A study of the details shows further that the results are reliable. The main efferent canals are lined with the epithelial membrane depicted in fig. 20. It may be seen that the membrane consists of a single layer of flattened cells that are separated by wide spaces across which abundant intercellular connectives pass. The cells are in general elongated in a direction transverse to the long axis of the canal, but polygonal cells also occur. The cytoplasm is granular and vacuolated, and quite without distinct boundaries. It passes insensibly into the intercellular connectives. The vacuoles vary in size and are irregularly distributed. In many cells the granules are scattered more or less uniformly through the cell, but quite commonly they are distributed in dense and pretty straight tracts which often extend along one margin. The nuclei appear to be all alike. They uniformly show the membrane,

nucleoplasm, and chromatin in the shape of granules or short pieces (doubtless a reticulum exists).

The term "cell" and the idea expressed by it are not altogether appropriate to the nucleated areas present in this membrane. The areas are everywhere united by abundant intercellular connectives, and merge very gradually into these, the cytoplasm often thinning away into reticulated films which then pass into the intercellular strands. Where the margin of the cell area is densely granular, the distinction between cell and connective is sharper (a figure inevitably represents the contrast between cell body and connectives as sharper than it exists in nature). The nucleated areas are frequently directly confluent, so that one and the same area may contain two nuclei (fig. 20). The membrane is actually of course a syncytium, but it is one in which the component cells permanently remain in a state of imperfect union. The regenerating epidermis passes through an essentially similar stage (fig. 7). The canal lining thus remains in a condition not so far removed from the mesenchyme as is the epidermis. Like the epidermis it may be regenerated from the mesenchyme, probably as Weltner ('07) maintains, largely from the granular amoebocytes.

When the membrane is examined with a comparatively low power, the nucleated cell areas appear to have distinct boundaries and to be independent cells separated by wide spaces. Fixation with sublimate may lead to the same erroneous conclusion. In fig. 21 the canal epithelium is represented as it appears when prepared from sublimate material. The cells are widely separated and have good sharp boundaries. The cytoplasm is finely granular and fairly dense. Almost no intercellular connectives are present. The absence of the connectives and the uniform dense granular appearance of the cells when comparison is made with a good alcoholic preparation such as that from which fig. 20 was made, must be regarded as artefacts due to the sublimate treatment.

There is good indication that the lining epithelial cells are contractile and of themselves bring about the diminution in bore of the canal. The canals certainly do diminish in bore, and greatly at times. Round the canals there are no fibre-like mesenchyme cells

arranged sphincter fashion. But the shape and arrangement of the lining epithelial cells suggests plainly that they are the closers of the canal. As I have said the cells lining an expanded canal are in general elongated transversely to the long axis of the canal. Very often the cell is so long and narrow that it is properly described as fibre-like (figs. 20 and 21). Mingled with such one finds other cells that are not greatly elongated and still others that are polygonal (fig. 21). I have examined some canals in which contraction had very materially diminished the size of the lumen. In these I found that a very large number of cells were either only moderately elongated or were polygonal. This is what one would expect to find if the epithelial cells do by contraction shorten and so tend to close up the canal.

In this connection it may be noted that in the case of contracted canals, when seen in cross section, the surrounding mesenchyme (collenchyma) cells are found to be greatly elongated and arranged in such fashion that they radiate outwards from the canal wall. The appearances suggest that as the epithelial cells are the closers of the canal, the surrounding collenchymal cells act as openers.

COMPARISON

It is well known that in a large number of sponges the dermal surface is covered and the canals lined with a single layer of cells (pinacocytes of Sollas) forming an epithelium. It was F. E. Schulze who in 1875 first established this fact. After demonstrating the presence of epithelia in *Sycandra* he showed in succeeding numbers of his classical "Untersuchungen" that the same or very similar structural conditions are found in a great variety of sponges. Schulze's conclusions have been confirmed and extended, for the same and other forms, by many observers. A review of the literature shows, however, that both Schulze and other observers have now and then, in this sponge or that, been unable to demonstrate the presence of distinct cells in the epidermal membrane. Possibly in some of these forms the epidermis is a continuous syncytium as in *Stylotella* and *Reniera*. With regard to the canal lining a number of recorded facts suggest

that loose epithelioid membranes such as I have found in *Stylorella* perhaps occur with some frequency in place of typical epithelia composed of polygonal cells fitting together neatly by straight edges. The covering layers of surfaces exposed to the water are certainly less uniform in sponges than was supposed some years ago to be the case. The hexactinellids in particular depart from the common condition. In these sponges, as Ijima's important discoveries seem to show, the covering layers in question can not be regarded as epithelia at all.

In the following sponges the occurrence of epithelia on the surface of the body, or lining the canals, or in both situations, is well established.

Calcarea. In *Sycandra* (Schulze '75) the dermal and gastral surfaces are covered with an epithelium composed of a single layer of flat polygonal cells which fit together neatly. In *Grantia* according to Dendy ('91a) the epidermis is a simple flat epithelium and the inhalent canal system is lined with a similar layer. In *Vosmaeropsis* too, Dendy ('93) finds that the epidermis and canalar lining are simple flat epithelia. In *Clathrina*, Minchin ('00) finds the dermal surface covered with flat polygonal epithelium cells, between which are intercalated the peculiar pore cells. In *Leucosolenia*, Dendy ('91 b) finds the dermal surface covered with thin flat polygonal epithelium cells. The more recent investigations of Urban ('06) show that while the flat cell is the common type, the epidermis also includes cells of other shapes, some cylindrical, some flask-shaped, the latter probably glandular. Minchin ('08) confirms Urban's account as to the variation in shape of the epidermal cells in this genus.

Carnosa. In *Chondrosia* and *Chondrilla* (Schulze '77 b) the canals are lined with a simple flat epithelium. In *Plakina* (Schulze '80) the dermal surface and canals are covered with a single layer of flat epithelium cells that are flagellated. In *Plakortis* (Schulze, loc. cit.) the conditions are similar except that the cells are probably not flagellated. In *Corticium*, Schulze ('81) finds that the dermal surface is covered with flat epithelium cells. The canals of this genus are lined in places, according to Lendenfeld ('94, p. 74) with columnar epithelium.

Tetractinellida. In Craniella and some others of the "Challenger" tetractinellids Sollas was able to distinguish epithelial cells. He does not state whether the cells in these cases were epidermal or canalar ('88, p. 36). In Geodia and Ancorina Lendenfeld finds ('94, p. 74) that the canals are lined in places with massive cells.

Monaxonida. Among the Clavulina Lendenfeld finds ('96) in Tethya, Suberites, and Polymastia that the epidermis consists of flat epithelium cells. In Vioa, Suberanthus, Astromimus, and Papillella he finds the canals lined with epithelium cells which in some cases are flat, in others high, the two varieties perhaps only representing different physiological states of the same elements. In Suberites, Thomson ('86) observed that the epidermis consisted of a single layer of small, polygonal, and apparently unequal cells. Among the halichondrine monaxonida the Spongillidae are perhaps best known. In these sponges (Ephydatia) Weltner ('96, '07) finds the epidermis and canal lining made up of flat epithelium cells (pinacocytes). Delage and Hérourard describe ('99, p. 176) the same condition as obtaining in Spongilla. According to Weltner the epidermal cells include the pores which would therefore be intracellular.

Keratos. In Aplysina (Schulze '78 a) the dermal surface and canals are covered with flat epithelium cells. In Spongelia (Schulze '78 b) the same condition occurs. In Euspongia and Hircinia (Schulze '79 a, b) the canals are lined with flat epithelium. In Aplysilla (Schulze '78 a, Lendenfeld '89) the epidermis and canalar lining are made up of flat cells. In Dendrilla and Halme Lendenfeld ('89) finds that epidermis and canalar lining are made up of flat epithelium cells that are flagellate. In Ianthella (Lendenfeld '89) the epidermis consists of flat cells.

Myxospongida. In Oscarella lobularis (Schulze '77 a) the dermal surface is covered with a single layer of fairly thick cells that are flagellate, and the canals are lined with a similar layer. In Halisarca Dujardini (Schulze '77 a) the canals are lined with flat simple epithelium.

In the *Hexactinellida* true epithelia appear not to be present either on the surface or lining the canals. A thin nucleated protoplasmic layer to be sure, has been known since F. E. Schulze's

examination of the "Challenger" collections ('87) to occur on the dermal and canalar surfaces in many of these sponges. Schulze regarded the layer as an epithelium, stating, however, that he was not able to detect the contours of the cells. Ijima ('01, '04) finds that such membranes do not consist of differentiated epithelial cells distinct from the underlying trabecular tissue (essentially a plexus-like syncytium of mesenchyme elements), but are produced simply by the flattening out of the superficial trabeculae. In some cases there is really no bounding membrane, since the general syncytium preserves at the surface its character as a reticulum. Schulze is inclined ('04, p. 202) to assent to Ijima's position and remarks "Es ist also wohl anzunehmen dass hier" (in the hexactinellids) "die Differenzierung der oberflächlich liegenden Gewebszellen zu echten epithelialen Pinakocyten unterblieben ist." The permanent condition of a hexactinellid would thus seem not to be far removed from that of a monactinellid (*Stylotella*) which is in process of regenerating its epidermis. Rarely it may happen that monactinellids linger permanently at this low stage of histological development. Suberanthus as recorded by Lendenfeld ('96, p. 172) seems to be a case in point: "Bei *Suberanthus flavus* ist die äusserste Gewebelage aus einer dichten, vielschichtigen Lage von unregelmässigen, massigen, multipolaren Zellen zusammengesetzt. Die äussersten von diesen sind auf der Aussenseite abgeflacht und bilden das äussere Epithel, unterscheiden sich aber sonst in keiner Hinsicht von den tiefer liegenden."

In the following sponges the records leave it uncertain what is the structure of the epidermis. In *Chondrosia* and *Chondrilla* (Schulze '77, pp. 18, 20, 23, 27) an epithelium does not occur on the dermal surface, which is covered with a thin, finely fibrous or homogeneous cuticular layer that is possibly formed by the fusion and metamorphosis of cells. In *Euspongia* (Schulze '79 a, p. 626) it is uncertain whether the epidermis is composed of distinct cells. In *Hircinia* (Schulze '79 b, p. 16) the structure of the epidermis is uncertain. Some observations would indicate the presence of distinct cells, others that no cell boundaries exist. In *Halisarca Dujardini* (Schulze '77 a, p. 38) the dermal surface is

covered with a peculiar layer, cuticular in appearance, probably formed by fusion of epithelial cells that undergo a gelatinizing metamorphosis.

The case of Reniera. In *Reniera aquaeductus* Metschnikoff ('76) thought that he was able with silver nitrate to demonstrate clearly cell contours in the epidermis. Keller a little later ('78) studied the histology of *Reniera* and found that the silver method did give him a well marked system of dark lines forming polygonal meshes. But in many meshes no nuclei were present, while in others they lay in the extreme corner of the mesh, or again, they often lay directly upon the lines. For these and other reasons Keller believed that the meshes did not represent epithelial cells but were to be looked on as artefacts. I agree with Keller in this interpretation. Keller watched the closing of pores in living preparations under the microscope, but does not mention any structure such as the pore membranes of this paper. He is perfectly right in discrediting the idea that the pores are closed by the contraction of surrounding muscle-like cells, and is substantially in the right in maintaining that the pores open and close through the movements (contraction) of a superficial layer. As to the nature of this layer Keller at that time followed Haeckel and believed that the outer part of the sponge body (what is usually called epidermis or ectoderm plus mesenchyme or mesoderm) is composed of a soft living sarcode (exoderm of Haeckel) in which certain structures, spicules and some cells, are imbedded. This conception became untenable with the publication of F. E. Schulze's "*Untersuchungen*" (1875-81).

Nature of pores. In the *Calcarea* specialized pore cells, porocytes, are described, the pore being a perforation of such a cell and therefore intracellular. The porocytes lie at the surface of the simpler olynthus-like forms (*Clathrina*), but in the more complex *Heterocoela* they are said to occupy a position in the walls of the flagellated chambers. The apertures at the surface of the *Heterocoela*, dermal pores, are usually thought of as intercellular gaps, *i.e.*, as apertures each of which is bounded by numerous cells of the epidermis (Minchin '00, pp. 27, 48). In the *Monaxonida* and other *Demospongiae* we find as in the *Heterocoela*,

apertures at the surface of the body, dermal pores or ostia, and apertures in the walls of the flagellated chambers, chamber pores or prosopyles. The accounts leave it uncertain as to whether the latter always have the same character (Minchin, loc. cit.). The assumption is usually made that the chamber pores are intercellular gaps. My own observations on this point are limited to the tetractinellid genus *Poecillastra* (Wilson, '04, p. 107, pl. 15, fig. 2). The material seemed to be well preserved and in sections it could easily be seen that the collar cells were wide apart and rested upon a bounding membrane which connected them, and which itself showed no cell boundaries. The chamber pores appeared as perforations in this membrane. If we look on the membrane as formed of thin extensions from the bases of the collar cells, the chamber pores here are equivalent to intercellular gaps. On the other hand there are investigators who think the chamber pores may be intracellular structures. Thus Evans ('99, p. 419, figs. 32, 33, 34) is inclined to believe from his observations on *Spongilla* that true porocytes exist in the walls of the chambers in this sponge.

In our ideas of the dermal pores too a certain vagueness prevails, which can only be cleared up by further investigations. The distinction between the actual aperture and the pore canal should, it seems to me, be borne in mind, although where the dermal membrane is excessively thin it may be that such distinction is in practice impossible. Usually the dermal pores are thought of as perforations of the dermal membrane, the two layers of epithelium being continuous round the margin of the pore. Where cell boundaries exist in the epidermis, such a pore, *i.e.*, the actual aperture, would have the nature of an intercellular gap and would not differ in its fundamental structure from an osculum. I conceive the pores in *Stylotella* and *Reniera* to be of this nature. Were the epidermis in these sponges divided up into distinct cells, I take it that the pores (comp. figs. 1, 2, 3, 11) would each be surrounded by several cells. The observations of several investigators, however, have inclined them to believe that the dermal pores are intracellular structures, perforations of cells that are comparable to the porocytes of *Calcarea*. In the very young,

recently metamorphosed *Axinella*, Maas ('93, p. 350, pl. 21, fig. 37) finds that the surface views of all his preparations speak for this interpretation. If this idea be true, the porocyte occupies the thickness of the dermal membrane, extending from the outer surface to the subdermal cavity. Delage in describing the recently metamorphosed *Spongilla* ('92, p. 398, pl. 16), discusses whether the pores be intercellular gaps or intracellular structures, and thinks they are probably the latter. The relation of the porocyte, provided it exist, to the dermal membrane as a whole would here be problematical, since according to Delage, when the pores appear the mesenchyme and the inner epithelial layer of the dermal membrane have not developed. Weltner ('07, p. 276) too is led by his observations on *Ephydatia* to regard the dermal pores as intracellular. He speaks of them as perforations of the pinacocytes and mentions that the latter can change their shape. It is evident that a more extended, comparative study of the point is needed. It is not impossible that beneath the appearances recorded by the above named authors will be found the structural conditions described here for *Stylotella* and *Reniera*. In passing it may be noted that both Delage and Maas figure the epidermis as without cell boundaries.³ If cell boundaries really exist, it is remarkable that they should not be visible in such thin membranes at a magnification of 750, the magnification at which Delage's figures are drawn.

Canal epithelium. The loose epithelioid membrane which I have found lining the canals of *Stylotella* cannot be an isolated structure. Several facts recorded in the literature indicate that it may possibly be a common type. From among these I may mention the following: Sollas ('88, p. xxxvi) describes the epithelium lining the cortical canals of *Pachymatisma* as "without definite cell outlines, but the contained protoplasm, however, is very admirably displayed, as a superficially extended film produced into innumerable fine, sometimes branching threads." "The thread-like processes of adjacent cells seldom appear to unite,

³ Delage, loc. cit., pl. 16, fig. 9 b, for *Spongilla*; pl. 21, fig. 5 a, for *Aplysilla*. Maas, loc. cit., pl. 21 fig. 37, for *Axinella*.

but terminate abruptly." The figure (pl. 34, fig. 22) given by Sollas indicates plainly that the canal lining in *Pachymatisma* is similar to that in *Stylotella*. Dendy ('93) finds that the cells of the canal epithelium in certain calcareous sponges, *Grantessa*, *Sycon*, *Vosmaeropsis*, are sometimes separated from one another by wide intervals. He regards this appearance as due to contraction, the cells having pulled away from one another. They may still remain connected, he says, in places by strands of protoplasm. Some of Dendy's figures (pl. 14, figs. 60, 62, 64) suggest that in these sponges too the canal lining may be of the type found in *Stylotella*.

NOTE—I am fortunately able to refer to a publication by Professor G. H. Parker⁴ that has appeared while the foregoing paper has been passing through the press. Parker, in the course of an interesting physiological study of *Stylotella heliophila*, one of the forms on which my observations were made, touches incidentally on the histology. Some of his conclusions differ from mine.

He thinks that the dermal epithelium is composed of polygonal cells. This conclusion rests on a study of sections which were apparently vertical to the surface and made from osmic material. The dermal layer is so thin that I do not believe it possible to learn much of its structure from such preparations. The same criticism applies to the conclusion that the dermal pores are surrounded by elongated spindle-shaped cells, myocytes, which act as sphincters. Surface preparations, such as those from which my figures were made, show that the pores are not surrounded by cells of this kind.

Parker finds that the canals are lined with a flat epithelium. In addition an abundance of myocytes surround the canals (and oscula) arranged like sphincters. "In some places in my preparations they seem to lie directly on the exposed surfaces of the canals and cavities that they bound as though they were merely elongated epithelial cells" (loc. cit., p. 7). It is

⁴G. H. Parker. The Reactions of Sponges, with a Consideration of the Origin of the Nervous System. Jour. Exp. Zool., vol. 8, 1910.

clear from this quotation that Parker has seen the same elongated epithelioid cells which I have described as lining the large efferent canals. My precise observations were limited to these canals, but I may say that I doubt if both an epithelium and an outer sphincter-like layer of myocytes bound any of the canals. Round the osculum the case must be different. Parker here finds a well marked sphincter.

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EXPLANATION OF FIGURES

1 Stylotella. Dermal membrane over subdermal cavity—from thick tangential section. *cc*, cells lining subdermal cavity; *ep.n.*, epidermal nuclei; *m.c.*, mesenchyme cells; *p.c.*, wall of pore canal; *p.m.*, pore membrane; *s.c.w.*, wall of subdermal cavity. $\times 1200$ (Zeiss 2 mm., oc. 6).⁵

2 Stylotella. Dermal membrane perforated by pore canals—from thick tangential section. Pores closed. References as before. $\times 1200$.

3 Stylotella. Dermal membrane perforated by pore canals—from thick tangential section. Pores partially closed. References as before. $\times 1200$.

4 Stylotella. Dermal membrane showing osmic-silver artefacts. From thick tangential section. $\times 1200$.

5 Stylotella. An oscular lobe was cut transversely. Part of cut surface is shown; canals have been closed in by newly formed membrane. *ch.*, choanosome; *col.*, collenchyma; *c.w.*, wall of canal; *c.m.*, newly formed membrane closing in the canal. $\times 85$.

6 Stylotella. Regenerating epidermis. Exposed surface two hours after cutting. $\times 1200$.

7 Stylotella. Regenerating epidermis. Exposed surface five hours after cutting. $\times 1200$.

8 Stylotella. Regenerating epidermis. From tangential section of new dermal membrane that has closed in a canal. Five hours after cutting. Body of figure is drawn at a focus below cut surface, and shows mesenchyme cells, *m.c.*; *p.c.*, space in mesenchyme, probably representing pore canal. At a different focus the epidermis, *p.m.*, is drawn where it roofs in the space. In such a position the epidermis presumably forms a (closed) pore membrane. $\times 1200$.

9 Stylotella. Regenerating epidermis. Exposed surface twelve hours after cutting. Pore canals, *p.c.*, now perforate the dermal membrane; small perforations of the epidermis, *per.*, occur. $\times 1200$.

10 Reniera. Successive stages in the closure of a pore. $\times 600$ (Zeiss D 4).

11 Reniera. Epidermis from an oscular tube. Walls of pore canals, *p.c.*, shown at a lower focus. Pore canals partially or completely closed in by pore membranes, *p.m.* $\times 1200$.

12 Reniera. Successive stages in the closure of a pore. $\times 600$.

13 Reniera. Dermal surface.—from an oscular tube. *p.c.*, pore canal of fig. 12, now closed in and contracted; *a-d*, cells in the mesenchyme. $\times 600$.

14 Reniera. Successive stages in the closure of a pore. $\times 600$.

15 Reniera. Successive stages in the closure of a pore. $\times 600$.

16 Reniera. Successive stages in the closure of a pore. $\times 600$.

17 Reniera. Three pores showing pseudopodial activity, at margin (*a*, *c*), or at margin of incomplete pore membrane (*b*). $\times 600$.

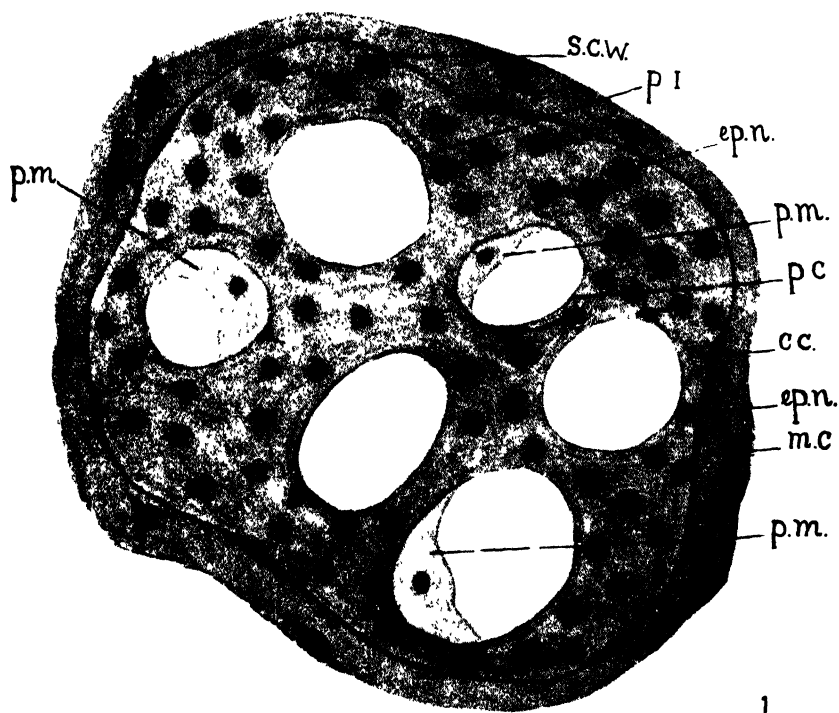
18 Lissodendoryx. Late stage in the closure of a pore. *p.c.*, wall of pore canal; *p.m.*, pore membrane, $\times 600$.

19 Lissodendoryx. Stage in the closure of a pore. $\times 600$.

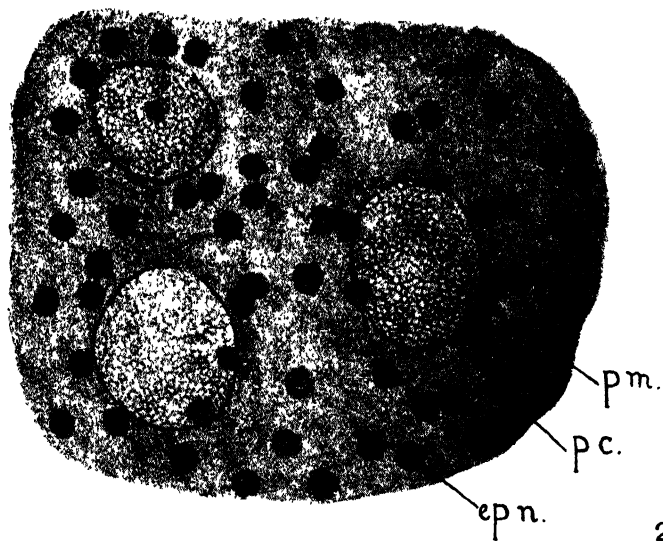
20 Stylotella. Epithelioid lining of main efferent canal. Alcohol fixation. $\times 1200$.

21 Stylotella. Epithelioid lining of main efferent canal. Sublimate fixation. $\times 600$.

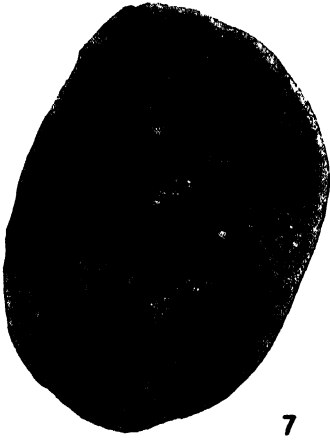
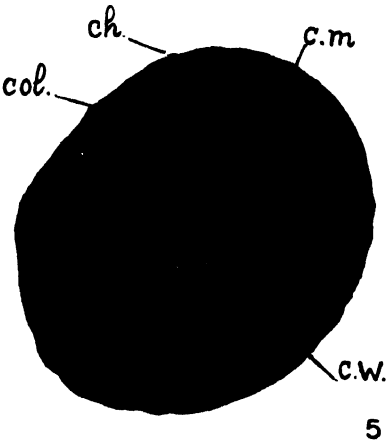
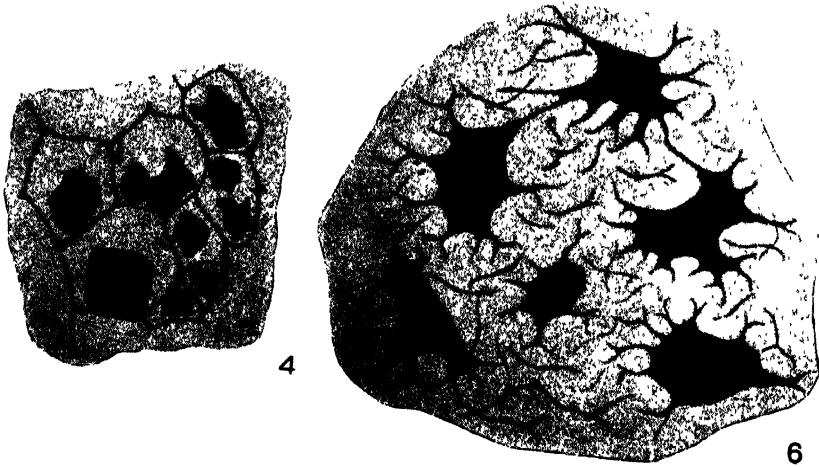
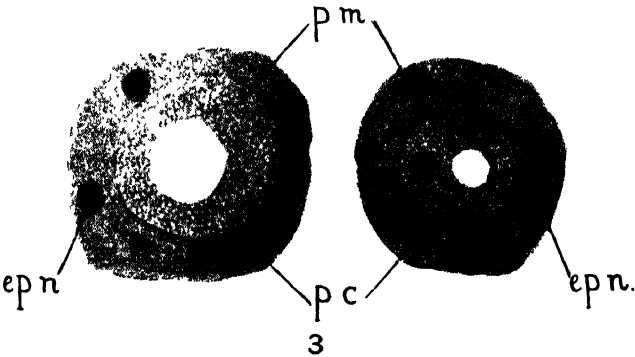
⁵ All figures have been reduced in reproduction by one-third.

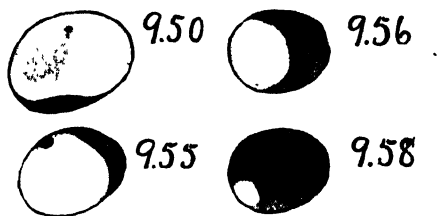
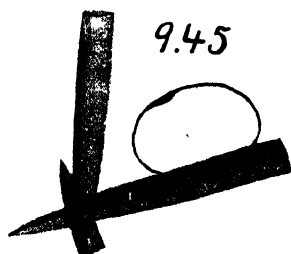
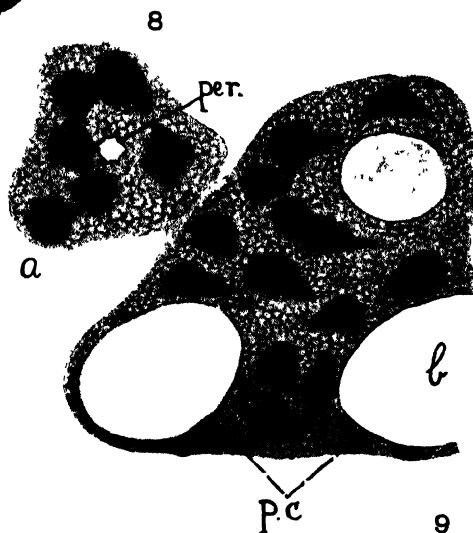
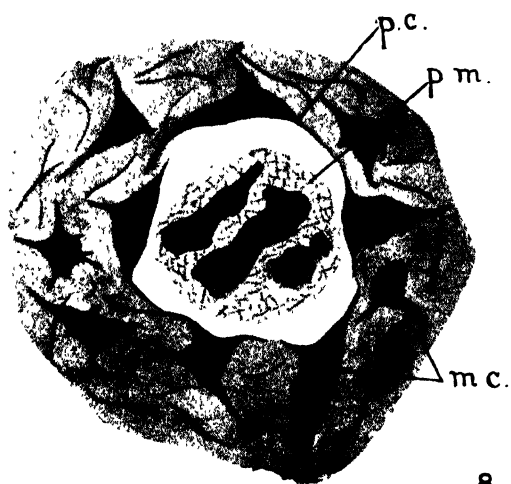


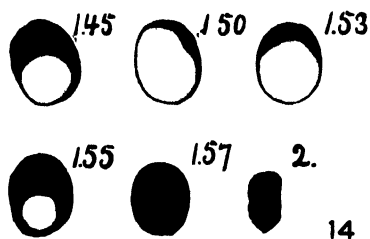
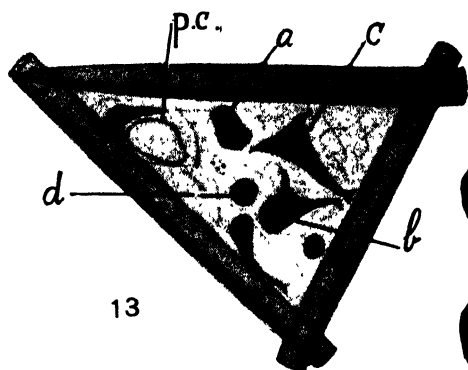
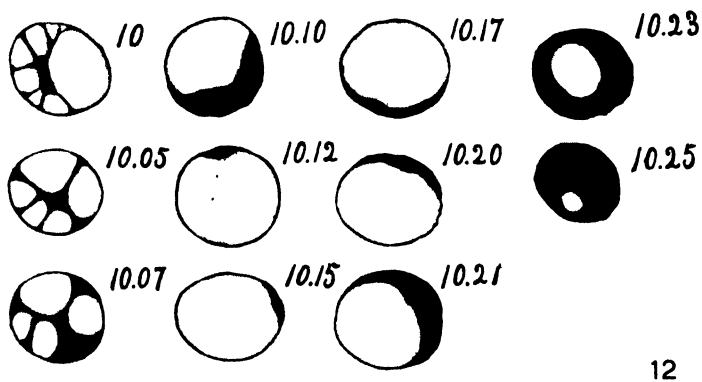
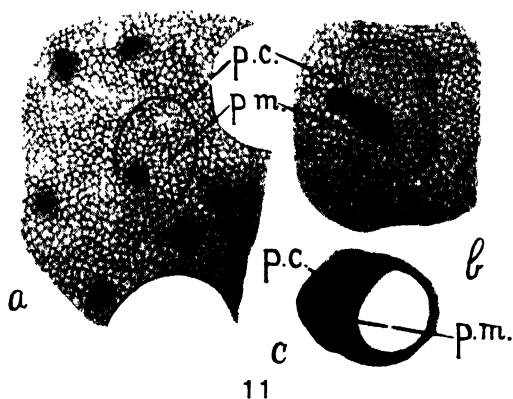
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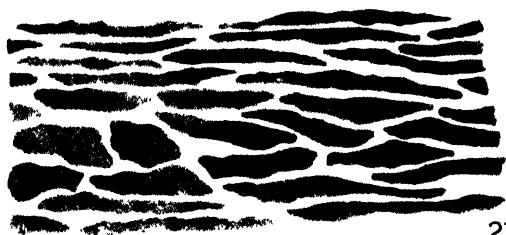
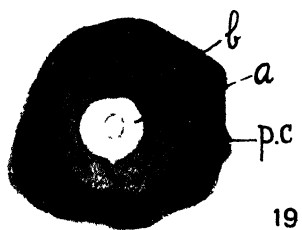
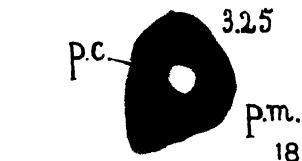
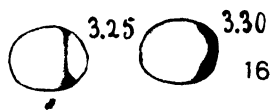
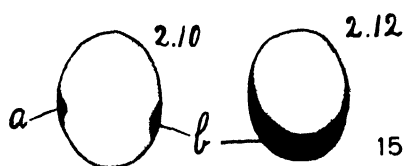
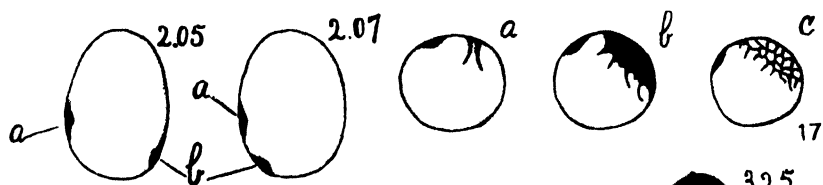


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AN EXPERIMENTAL DETERMINATION OF THE SPEED OF MIGRATION OF SALMON IN THE COLUMBIA RIVER¹

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TWO FIGURES

Certain fishes, like some birds, carry out extensive migrations, but unlike birds, their movements are hidden from direct observation. Commercial fishermen dip their nets into the waters and learn to know many of the movements of fishes by the character of the catch. Certain fishes travel in great schools, and this tendency to herd together furnishes an easy method for following, especially those schools that swim near the surface. Presumably the migratory movement serves one of two purposes; either it is a means for providing food, or it brings fishes to the spawning ground.

The method of following the movement of fishes by the quantity of the catch is at best crude. One has no assurance that the school from which the catch is made in one locality at a given time is identical with that from which another catch is taken, even in approximately the same locality and at a nearly related time. For this reason it is almost imperative that one shall identify the individual fishes observed in order to determine their movements from one locality to another. Following this line of reasoning, Rutter² undertook to brand migrating Pacific salmon in the Sacramento River in 1902. This seems to have been the first effort at marking individual salmon with a view to determining their migrations in our North American waters.

¹ Published by permission of the Commissioner of Fish and Fisheries.

² Rutter: Bull. U. S. Bureau of Fisheries, 22, 122, 1902.

The Pacific salmon is an unusually favorable fish upon which to determine the factors in migration. It has been known for a number of years that these fishes spawn in the cold waters of fresh water streams, usually in the mountains. The eggs are hatched and the young are developed to the fingerling stage and then migrate down the rivers to the sea. In the sea they feed a number of years until maturity. When maturity is reached they reënter the estuaries of the rivers, ascend the rivers to spawning grounds and deposit their eggs. In the case of all the species of the genus *Oncorhynchus* the fishes die after they once spawn. They do not again return to the salt waters of the ocean.

When a salmon once enters the fresh water rivers, it, seemingly, makes a pretty direct run to the spawning grounds. This run often extends over several hundred miles and may consume as much as two or three months of time. When the fish enter the rivers they are in the very finest physical condition. Their muscles are developed apparently to the fullest extent and their tissues as a whole are loaded with nutritive material, *i.e.*, fat. This is the result of the long months of feeding during the life in the ocean. Apparently the ocean furnishes rich feeding grounds and is visited for the distinct purpose of supplying a more favorable condition of nutrition. When the fish enter the fresh waters for their long journey they stop feeding absolutely, a fact that is well known for the Atlantic salmon from the work of Miescher and of Noel Paton. These facts in the case of the Pacific salmon have been determined by the observations of numerous investigators of the United States Bureau of Fisheries. Rutter particularly has shown that the salmon not only does not eat but that the digestive tract diminishes sharply in size during this fasting period. I have confirmed Rutter's work for both the Sacramento and the Columbia River regions.

This migration of salmon in fresh water is a period of strong activity. The fishes make their way up the streams against strong rapids and sharp water falls. Before passing the latter obstructions salmon will oftentimes leap the falls in many unsuccessful attempts, even jumping to the extent of six or seven feet in height and against the known swiftness of the water under such condi-

tions. This tremendous output of energy can only take place at the expense of nutritive substance on hand when the journey is begun.

A still more fundamental process is occurring during the migration, namely, the rapid development of the reproductive tissues. When the salmon enter the mouths of the rivers, especially earlier in the season, the reproductive organs are very small in size and immature in development. As the journey progresses the reproductive organs in both sexes grow rapidly and the reproductive cells approach maturity of form and structure. This growth of new tissue in the absence of an income of food represents a remarkable physiological process. Such growth can take place only at the expense of materials on hand, and to produce new tissues requires substances more complex than the mere fats, which might possibly account for the production of the energy of motion. We have, therefore, in this animal two lines of physiological activity which lend peculiar interest to the subject of nutrition in the absence of food, namely, first, the source of the dynamic energy expended by the animals, and second, the source and character of the nutritional changes which result from the development of a special set of organs at the expense of other portions of the body. It is obvious that the question of the rapidity and of the intensity of the energy changes in these processes are the all-important factors. In order to secure information which would help to elucidate those factors, it became necessary to determine as accurately as possible the details of the migration of salmon. It seemed desirable to determine the speed of the migration, the total time consumed by the fish in the journey, and the detailed character of the migration, all with a view to determining the intensity of the energy put forth in making the journey.

In order to subject the questions at issue to a test, I arranged a marking experiment on fish secured in the lower Columbia River. It seemed that the only way to get accurate information would be to mark individual fishes in such a way as to be absolutely certain of their identification. My plan was to secure the live fishes, mark them with metal tags and turn them loose in the river again with the hope that they would be re-taken. The numerous com-

mercial fishery interests in the lower Columbia River gave abundant opportunity for the recapture of individual fishes, notwithstanding the broad expanse of territory necessary to be covered. Rutter marked his fish on the Sacramento River by a method of branding with a hot iron, similar to that used for marking cattle on the plains in early days. Of some 150 fish branded by him, only three were afterwards re-taken an the Government Fisheries in the lower head water of the river.

On August 4, 1908, I secured and marked with metal tags 59 fish. These were immediately liberated in the river in good condition and in each case quickly swam out of sight. The fish were secured through the kindness of Superintendent Nicholay Hansen of the Chinook, Washington, Fish Hatching Station. Superintendent Hansen generously furnished transportation and facilities for taking and marking the fish. The fish were taken from the Washington State Trap which is located just above Sand Island about eight miles from the mouth of the Columbia River. This location was peculiarly favorable to the execution of the experiment. In the first place the spot is well within the mouth of the river, so that the average salinity of the water is only mildly brackish. It is also on the border land between the two great fishing fields utilized by the traps on the one hand and the gill nets on the other, thus giving the liberated fish a fair chance for a successful run. The location promised opportunity for determining whether or not the fish might migrate back and forth through the estuary with the ebb and flow of the tides, since Rutter has advanced the theoretical view that such is the case. The later details will show that the location was especially fortunate with reference to this particular point. The salmon were run from the trap into a large live car used by the station. They were marked one by one from this car.

The apparatus adopted for marking the fish in this experiment was the aluminum metal stock marking button used for marking domestic animals,— sheep and cattle. The button is adapted for insertion in the ear of sheep or other animals and can be riveted together in a way that makes it absolutely impossible to break it apart except by actually tearing it from its location. The

aluminum is light and furnishes sufficient surface for the stamping of numbers, initials, or other matters of identification. The tag was made of two pieces on the general principle of a Yankee button. The first piece consisted of a circular disc forged on to a hollow shaft some 7 mm. in diameter. The disc of this piece had a serial number stamped upon it. The second piece was similar to the first except that the disc was forged on a solid rod or rivet. The disc of this piece carried the legend "U. S. Fish." When the rivet of the second piece was inserted into the shaft of the first piece and compressed, the aluminum filled the cavity so as to make it impossible to separate the two.

THE DETAILS OF THE MARKING

The salmon is a gamey fish. When taken in a dip net it struggles violently to escape. By skillful management a salmon can be held just under the surface of the water in such a way that its struggles will produce marked fatigue. When a fish is thus fatigued it will remain quiet for some seconds. In this work advantage was taken of that fact and the instant the salmon stopped struggling it was lifted out of the water, grasped by the base of the tail, swung free of the net, and laid gently on the measuring board. In this position the tip of the nose was brought up against the vertical end of the measuring apparatus, and the length read off and announced to the recorder. The next step was to attach the marking button. This was done by puncturing a hole in the caudal fin into which the button was inserted and riveted. The whole process required less time than it takes to describe it.

The salmon stood the handling very well. They were immediately released overboard in the direction of open water. If there was the slightest question as to asphyxia, the fishes were first released back into the car and later, when they had fully recovered, were turned into open water. The fishes of this series came through in exceptionally good condition.

A DISCUSSION OF THE INJURY INFLICTED IN THE MARKING PROCESS

No physical injury is imposed upon the fishes up to the point where the 7 mm. hole is punched in the tail for the insertion of the marking button. This injury is relatively insignificant. True it produces a transient stimulus to the skin which leads to physiological reflexes for the moment. If the button is carelessly inserted so as to continually compress the tissue of the caudal fin, this may lead to further stimulation of a certain degree.

A factor of far more importance than the direct physical injury is the possible asphyxiation that results during the handling of the fish in air. When a fish is taken out of the water, the water quickly drains from the mouth cavity and from over the gills, thus exposing the latter to air. When the air comes in free contact with the gill filaments, even better aëration may occur than when the fish is in water. The trouble comes when the gill filaments no longer supported by water adhere together in a mass. Under these conditions asphyxiation takes place rapidly. The anatomical arrangements of the gillcovers and gills in different species are, for the above reasons, largely responsible for variations in the rapidity with which asphyxiation occurs. In the salmon one cannot but note the endurance of the fish against asphyxiation and the ease with which this condition may be removed by artificial respiration. A fish can endure a considerable degree of asphyxiation without any notable evil effects unless that degree be carried to the point which results in loss of function to the respiratory center.

A DISCUSSION OF THE INFLUENCE OF HANDLING ON THE MIGRATION OF THE SALMON

A most important question that arises in this experiment is this: What effect will the handling have on the future course of migration, and the manifestation of the migratory instincts in the salmon? It will have little effect for the following reasons:

Recently Edinger³ has called attention to the fact that the low form of the brain of the lower vertebrates must be taken into consideration in judging the reactions of such animals to stimuli. The salmon, for example, should be considered in view of its biological position in the animal series. Its brain is very simple in type. The cerebral lobes are small and the cortical structures of relatively slight complexity. If the salmon possesses any association tracts they are very simple. Such a low form of brain cannot execute very complex reactions. There is not the necessary anatomical machinery. The brain and spinal cord are capable of carrying on only the usual reflexes of muscular movement, circulation, respiration, etc. It is to be assumed that the injury to the skin, as for example punching the hole through the tail for the marking button, leads to little more than the reflex of muscular movements. As a matter of fact direct observation shows that these muscular responses are relatively slight, and include little more than the motions of swimming. There is in addition a transient inhibition of the respiratory movements. Judging from the results of other experiments, one may assume that there will be some slight disturbance in the coördination of the circulatory apparatus. These more or less complicated reflexes disappear within a few minutes at most.

Those conditions which lead to the migration of salmon are the chief directive stimuli at the migration time. They supercede all other stimuli. In comparison with them the effects of transient stimuli, such as cutaneous lesions, etc., are insignificant. Large numbers of fish taken in the upper waters of the Columbia River are injured in one way or another. Some of them have received lacerations many fold greater than that inflicted upon the fish for the purposes of marking. Yet these fish are forging ahead toward the spawning grounds with apparently no digression.

³ L. Edinger: Ueber das Hören der Fische und anderer niederer Vertebraten; *Zentralb. f. Physiol.*, 22, 1, 1908.

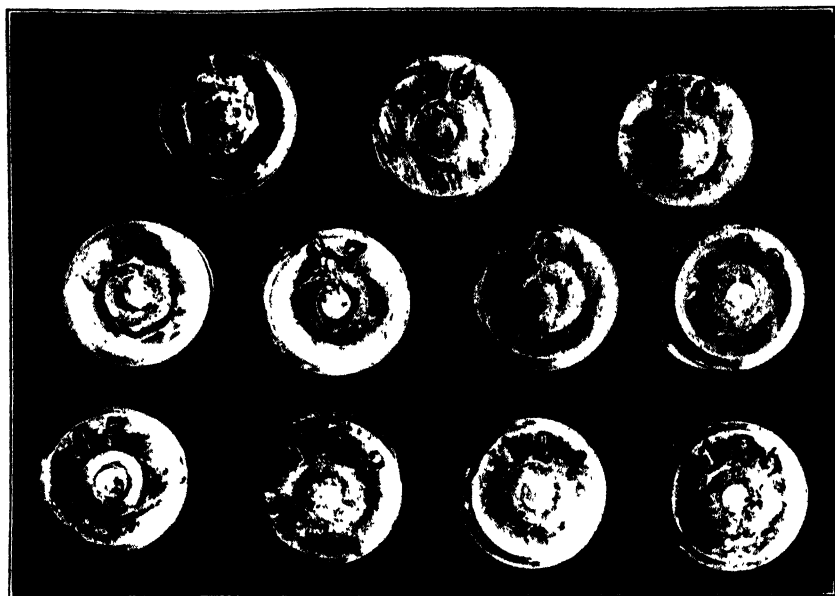


Fig. 1 Photograph showing the corrosion of the aluminum marking buttons recovered from the marked fishes. The tags are not included for the fishes re-taken within a few days.

was spent in water as salty as that represented by the region of Canby Light.

Chinook number 123 was out thirty-one days and traveled up stream a distance of only fifteen miles, requiring two days for the journey. Its button was the second deepest corroded of the series. The corrosion is evidence that its bearer spent much if not most of the time below the point in the river where it was liberated. This evidence is second in importance only to actual capture of the fish below the region.

One Chinook, number 113, was recaptured, six miles down the river from the point of liberation. As it was out only six days no corrosion occurred.

The silver salmon made the longest runs, five of the six specimens re-taken having traveled a distance of two hundred and ten miles. Three of the five fishes, numbers 76, 89 and 97, each bore

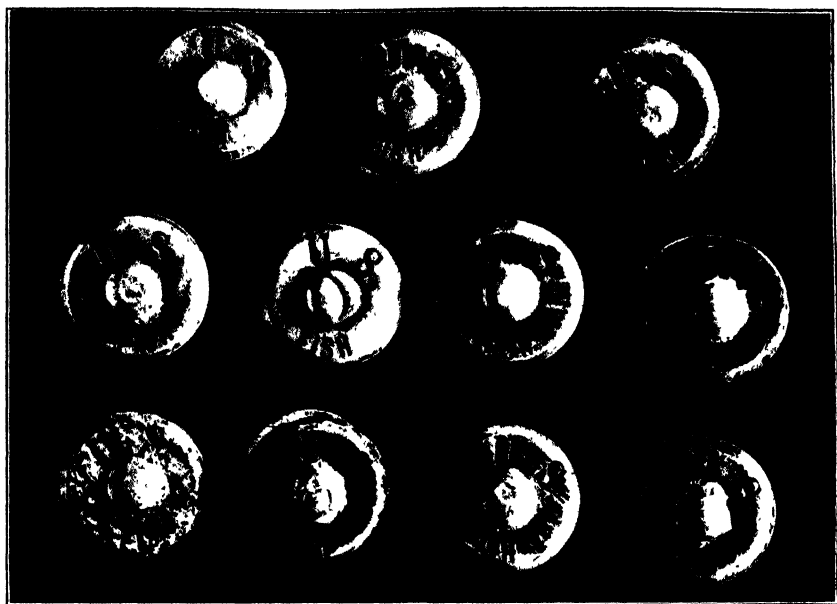


Fig. 2 Photograph showing the obverse faces of the aluminum buttons pictured in fig. 1 The order of arrangement is the same in the two pictures.

marking buttons that were extensively corroded. These fish made the entire journey at an average speed of from 6.36 to 7.50 miles per day. On the basis of the evidence that corrosion furnishes, these individuals must have spent some time in brackish or salt water.

Silver salmon numbers 79 and 75 bore buttons that were in the first case not corroded, and in the second only slightly corroded on one side. From the line of evidence which has been given consideration one must infer that these fish did not spend much time at or below the point where they were liberated.

Silver salmon number 87 is a decided exception in the list. This fish was re-taken only seventy miles up the river, yet it was out a total of fifty-seven days. The marking button does not present evidence of long contact with sea-water. It is slightly corroded on one surface but not more than would occur in slightly brackish water.

Of the 18 steelhead that were marked and liberated 5 were re-taken. Number 116 was caught four miles down the river about four hours after it was liberated. The remaining steelhead were caught, one in the region of the point liberated, one seventy miles up the river, and two were re-taken two-hundred and ten miles up the river. Of the last two, one, number 124, was out thirty-three days and bore a button that showed only slight corrosion. The other, number 98, was out fifty-two days and its button showed marked corrosion. Evidently the former spent little time in brackish water, while the marking button of the latter indicates a long contact with sea-water. The steelhead number 125, taken seventy miles up the river after thirty-five days, shows a history of contact with salt water similar to that of number 98.

THE AVERAGE SPEED OF MIGRATION

This experiment was launched in tide water. Hence the speed of migration is influenced by the factor of acclimatization of the sea run salmon to fresh water. Undoubtedly a very much higher speed is attained in fresh water than is accomplished in making the journey through the tidal region. A number of instances have been given to show that the fishes spent much time in the brackish water after their marking. It is safe to assume that salmon travel at a fairly uniform speed in fresh water when different individuals are compared under similar conditions. Inspection of table 1 suffices to show that either the assumption is untenable or that a number of the salmon have not made direct runs up the river. Undoubtedly the latter represents the facts in the case, and the corrosion of the marking buttons is corroborative proof. If the speed of the fishes is supposed to be uniform through the group and is computed on the basis of the average made by those representing the highest in the list, then, as table 1 shows, there will be for each fish, a number of days unaccounted for. In some cases, numbers 98, 125, 123 and 87 the unaccounted for days amount to 24, 26, 29 and 48 days respectively. These are the days which represent the period of adjustment of the

fish when it passes from the sea-water to the fresh water at the mouth of the river.

Rutter⁴ has advanced the theory that the salmon makes the journey through tide water by running up stream during the ebb, and down stream during the flood tide, that is, the fish stems the current during the flow of each tide. Rutter arrived at this conclusion by following the variations in the catch of the fisheries at the different towns along the San Francisco Bay and Lower Sacramento River. The theories of Rutter explain in part the movement of salmon in tide water, but tidal currents are not sufficient alone to account for the movements. A much more important factor is the condition of water as regards the content of salt. Salmon are delicately responsive to stimulation by the variation in the degree of admixture of sea-water and fresh water in the tidal area.

The present observations show a very much longer time spent by salmon in the tidal waters of the Columbia River than that deduced by Rutter for the Sacramento River. It is safe to conclude that the salmon spend not less than thirty to forty days in passing the tidal area of the lower Columbia River. When once through the tidal area they make the journey up the river at an average speed of not less than seven and one-half miles a day.

⁴ Rutter: Bull. U. S. Bureau of Fisheries, 22, 122, 1902.

CYTOLOGICAL STUDIES OF CENTRIFUGED EGGS

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ONE HUNDRED AND NINETEEN FIGURES

EIGHT PLATES

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1. CENTRIFUGING THE EGG OF CUMINGIA

When I began the study of the effects of centrifuging the eggs of *Cumingia tellinoides* in the summer of 1907, my object was to compare the results with those already obtained by Lyon and myself on the eggs of the sea urchin. At that time the observations of Conklin and of Lillie had drawn attention to the rôle of the visible materials of the egg as organ-forming. Although no evidence of the formative nature of the visible substances in the egg of the sea urchin could be obtained by the centrifuging experiment, it did not follow that in eggs with a precocious type of cleavage, such as Conklin and Lillie studied, visible organ-forming materials might not be present. I therefore took up the examination of *Cumingia* to test the question in the group of molluscs that shows the characteristic precocious type of development. In addition, I wished to study the effects on the karyokinetic division of the egg; for, it became manifest at once that the centrifuge furnishes an instrument of wonderful delicacy by means of which the parts of the egg may be shifted in regard to each other without destroying the power of development of the egg. At that time I was hopeful that at some stage in the karyokinetic process it might be possible to move the chromosomes, and in this way study the influence of the chromosomes on development. With this end in view every stage of the egg from the time of disappearance of the germinal vesicle to the four-cell stage has been centrifuged, but without in any case affecting the separation or scattering of the chromosomes. This attempt has consumed a good deal of time, and while the results are negative so far as the separation of the chromosomes is concerned, other important questions have arisen and some light has been thrown on the nature of the karyokinetic process.

I wish to express to Professor Gilman A. Drew my obligations for bringing to my attention the possibilities of the *Cumingia* eggs, and the method which he had worked out of procuring them in abundance. During June, July and August, when this little bivalve is brought in from the mud in which it lives and put into dishes of water, it begins after about half an hour or less to set free its eggs or its sperm, according to sex.

When collected, the animals were generally put into a tumbler and brought in dry. It appears that a short removal from the sea-water excites them to egg laying, but the same process will also occur if the animals are brought in with the mud in which they live. The males shed their sperm first. The females lay their eggs even when no sperm is present. It seems that the presence of sperm in the water may incite the females more promptly to deposit their eggs though the presence of the sperm is not necessary. The eggs are small and very numerous if the female is large, and if she has not previously spawned. Early in June fewer individuals lay eggs, and the eggs produce fewer normal embryos. Later in June and especially in July the egg-laying season is at its height, while in August fewer eggs are again obtainable. It is noticeable that these late eggs show signs of parthenogenesis. The polar bodies are extruded and an irregular cleavage often takes place.

I have already published two preliminary notes touching on some points concerning the centrifuged egg of *Cumingia*, but many of the results appear here for the first time.

The effect of centrifuging on development

When the egg is laid (plate 1, fig. A) the polar spindle is present in its middle (plate 2, fig. M). If sperm has been added to the water, before egg laying begins most of the eggs will have been entered by one (or more) sperm. After centrifuging the egg has the appearance seen in plate 1, fig. B; plate 2, fig. N. The oil is driven to one pole, and the heavier pigment and yolk to the other. The broad middle region is occupied by the clear protoplasm. The details of the polar-body formation and cleavage will be given later. Here I need only say that during these processes the enforced distribution of the materials may become altered, not in consequence, as is sometimes implied, of the remixing of the materials of themselves, or returning to their original position, but because of the movements of the karyokinetic spindle, and because of the process of cell division, plate 1, fig. F. Despite these changes the centrifuged materials often remain in large part segregated at the time of division, and, in consequence,

the yolk and pigment may pass entirely to the small cell, or, in other eggs, the small cell may contain the oil, or else only the clear protoplasm of the egg.

The development of the swimming trochophore (plate 3, fig. I) takes place in about 24 hours. If the eggs are ripe and the conditions favorable, the trochophore begins to flatten during the next 12 hours and after 48 hours (plate 3, fig. VI) has become a typical veliger (plate 3, fig. XII, XIII). By no means all sets (eggs from one individual) reach this stage. Even in the best part of the season not more than half of the sets become veligers and the others remain in the swimming trochophore stage until they die. This is the more surprising since the eggs segment with the precision of clockwork in nearly all of the sets, and the early stages of development appear to be normal.

This failure of many sets to produce normal embryos has added to the problem an unforeseen difficulty, which was greatly increased when it was found that centrifuged lots rarely produced normal veligers, although here too the early stages were entirely normal. Occasionally, however, a centrifuged set would produce many veligers and sometimes as many as its normal control. This result showed that centrifuging, in so far as it segregated the visible materials, was not the cause of the failure of most sets to develop. Convinced by this evidence that some other condition than the redistribution of the materials was responsible for this great majority of failures, I have spent a large part of two summers in the attempt to discover why the centrifuged eggs usually go badly, and why they occasionally develop as well as the controls. I have found an answer to these questions and the means of avoiding the trouble, as the following description of the experiments will show.

Polyspermy is a common cause of abnormal development. Eggs of an individual were put into small dishes and each set fertilized by the sperm of a different male. All produced abnormal embryos. As a control some of the eggs were put into a large dish of water and a little sperm added. They developed normally even when they were transferred to smaller dishes after fertilization. Sea water from the ocean gave better results than sea water

from the tap. Keeping the eggs cool gave no better result than warm temperatures.

It was found that a few drops of dilute sperm gave better results than when more sperm was added. Mixing the sperm of several individuals gave better results, on the whole, than sperm from a single individual, although one or more individuals gave as good results as the mixed sperm. This means that in mixing the sperm the chance of getting good sperm was insured. Individual females gave different results even when the other conditions were the same; showing that some eggs are better than others.

The eggs of seven females were centrifuged separately, and control sets of each female were kept. All of the centrifuged eggs went abnormally, while several of the controls gave normal embryos.

The eggs of nine females were separately centrifuged and then fertilized. Controls of each were kept. Only a few trochophores appeared in the centrifuged lots, while all of the controls were normal. Also seven lots of the same eggs were fertilized after they had stood 40 and 80 minutes; these went abnormally. Similarly five sets centrifuged produced abnormal embryos, whether centrifuged 75 turns of the handle or only 25 turns. The controls, however, were normal.

Six sets were centrifuged; in each, one part 25 turns,¹ the other 50 (or 100) turns. All went abnormally except one set which produced swimmers both in the 25 and 50 turned lots. The controls were all normal. Evidently certain individuals behave differently from others.

The preceding experiments suggested that something must occur in handling the eggs that is injurious to development. I put, therefore, whole animals in the water centrifuge and rotated them for 20 minutes. At the end of that time it was found that the ripe eggs in the animal had been perfectly centrifuged. If such animals were put into water they laid there their centrifuged eggs. Sperm was then added in small amount. In two

¹ It takes $3\frac{1}{2}$ minutes for 100 turns; fewer turns in proportionate time. Bausch & Lomb's hand centrifuge.

lots the eggs developed well. Here at last was a clue for further examination. Something in the handling of the eggs after they had been laid may have caused them to develop abnormally. I put some eggs in one of the centrifuge tubes and turned the handle only five times very slowly. This sufficed to throw most of the eggs to the bottom of the tube. As a control, eggs of the same individual centrifuged 25 turns were used. Without exception all went abnormally. Here the eggs had only been centrifuged by five turns of the handle, yet were injuriously affected. The result showed that something else than the centrifuging was responsible for the results.

Experiments with the eggs of the sea-urchin had shown me that if the eggs are thrown down suddenly on the centrifuge the membranes may be destroyed. The presence of a few drops of gum arabic (in sea water) at the bottom of the tubes prevents the eggs from becoming closely appressed and also helps to keep the membranes intact. I allowed eggs of *Cumingia* slowly to settle to the bottom of the centrifuge tube in which a drop of gum arabic had previously been placed. But in order to get the eggs out of the tube they must be rather roughly treated. Six lots so handled centrifuged only six turns, gave abnormal embryos. The gum arabic was a rather thin solution in the last experiment and the eggs sank into it. Thicker gum was used and the eggs removed carefully. All six lots developed normally. The gum solution was again used in six lots and abnormal embryos developed.

If, as the experiments indicate, the eggs are damaged by the removal of the membranes, the same results should follow if, instead of centrifuging, the eggs are simply squirted in and out of a pipette. Six separate lots, so treated, gave only abnormal embryos.

Eggs were allowed slowly to sink in the centrifuge tubes and were then roughly squirted out. They went abnormally. The controls were normal. Other eggs roughly handled also went abnormally.

These experiments show plainly enough that abnormal development is brought about by any handling of the eggs that is a little rough. The most plausible explanation is that such treatment

removes the membranes. Whether this is the way in which the result is reached or not, the facts show why the centrifuged eggs failed more often to produce normal embryos than eggs not handled.

The experiment in which the whole animal was centrifuged with its included eggs seemed to show that the separation of the materials of the eggs was not the cause of abnormal development. In order to test this conclusion further a large number of animals were tried. In some cases they were allowed to lay a few eggs (controls) before placing on the centrifuge, but in most cases the females were put on the centrifuge before they had deposited their eggs. An equal number of other individuals were allowed to lay their eggs undisturbed, and the proportion of normal and abnormal lots in the two series compared. The entire animals were centrifuged for 20 minutes on the water centrifuge. Of the six lots all produced normal trochophores.

When some animals had begun to lay, they were opened and pieces of the body containing the eggs put into the tubes of the centrifuge and revolved for 50 turns. When examined the eggs were found to be well centrifuged. The body was torn open and the eggs, set free, were fertilized. In each of the four lots many normal embryos were produced.

It will be observed that these eggs had not come in contact with sea water, hence no opportunity for the swelling of the membrane was afforded, and it seems probable that it may have been retained during the process of centrifuging.

Again four females that had begun to lay were opened and their bodies put into the centrifuge tubes and revolved 50 turns. The eggs were found to have been well centrifuged. They were fertilized and each set produced many normal embryos.

In this and in the last set also those eggs that did not develop appeared not to have been fertilized.

Nineteen females in the shell that had just begun to lay (in water containing some sperm) were centrifuged, twelve for 20 to 25 minutes; seven for only a few minutes. All of the eggs were well centrifuged. The animals were opened and the eggs fertilized. All the dishes contained normal embryos.

Four females that had begun to lay (in water containing sperm) were centrifuged in the shell for 10 minutes. They were then put into water where they began to lay in a few minutes. All of the eggs were seen to have been well centrifuged. All sets produced normal swimmers. In some cases the eggs were transferred to other dishes. These did not develop so well. About thirty-two other individuals gave similar results. In addition there were some sets that produced abnormal embryos. Comparison with lots composed of the same number of normal individuals showed that there were as many lots of the centrifuged eggs in which normal development took place as of normal eggs. *It is evident that the abnormal development so frequent in lots of eggs centrifuged after deposition has nothing to do with the effects of the centrifuged on the contents of the egg, but is the result of the handling of the egg after contact with sea water.*

The localization of the visible materials of the egg

Despite the fact that the yolk, pigment and oil are sometimes shifted before cleavage begins, the yolk remains in many cases either in place or, if shifted, moves as a mass (plate 1, figs. C, F, and plate 2, fig. P, Q). This holds also for the pigment and oil. When the egg divides the small cell may, to all external appearances, be made up almost entirely of the yolk (plate 1, fig H), since in size this cell corresponds approximately to the amount of yolk precipitated. Three eggs in the two-cell stage are shown in plate 1, figs. G, H, I. It will be noticed that in all three cases the first cleavage passes through the pole where the polar bodies lie. In the first figure, the smaller cell is composed mainly of the oil cap; in the second figure of the pigment (and the yolk within); in the third figure, the small cell contains oil, pigment, and yolk. Three four-cell stages corresponding to the last, are represented in plate 1, J, K, L. In the first the smaller oil-bearing cell has divided into two equal parts, as in the normal egg; while a third small cell, containing pigment, is budded off from the larger cell. In the next figure these conditions are reversed. In the third figure L (turned over as compared with I) the small cell has

divided, so that one of its products contains pigment, the other some oil, and at the same time the large cell has budded off a small cell rich in oil. It is important to note that the relative sizes of these blastomeres are the same as those of the normal egg, and that no variation in size takes place as a result of the different kinds of materials contained in them.

I was anxious to isolate eggs in which all the yolk, plate 1, fig. H, or oil, fig. G, was known to be present in a definite part of the egg; for it was conceivable at least that only those eggs develop normally in which the induced segregation corresponds with certain polar relations of the egg. The normal distribution of yolk, oil and pigment makes such an interpretation highly improbable, nevertheless I wished to get definite information on this point. Therefore in the summers of 1907-8 I made more than 1000 isolations with no result; since the sets themselves from which these were taken went for the most part abnormally. At this time the season was almost past and even normal eggs isolated produced abnormal embryos. In 1909 I again returned to the question after I had found that centrifuged eggs from centrifuged females often produced normal embryos. Although not nearly so many eggs were isolated, in all combinations, I failed to get normal sets owing in part to the handling, and in part to the fact that even in good sets some eggs go abnormally. I soon gave up these laborious attempts to isolate single eggs; for, I found that in the living animal it was quite easy to see in all stages up to the trochophore and even the veliger, the position of the pigment or of the oil. Sections of eggs up to and later than the gastrulation stage also showed that the yolk might be present in any part of the egg and normal gastrulation occur. I have examined hundreds of such sections but it did not seem necessary to give figures of them.

A series of figures of the living trochophores and veligers are shown in plate 3, figs. I-XV. The pigment is red, the oil is bluish. It will be seen that these materials may lie in any part of the embryo without producing abnormal development; figs. II-V; VII-XI; XIV-XV. *It follows that none of the visible materials of the egg of Cumingia are essential to the development of special parts of the embryo.*

This result meets a criticism that might be made of mass results in the absence of isolation experiments, namely, that only those embryos develop normally in which the direction of centrifuging coincides with the poles of the egg. Since in most sets there are some, often many abnormal embryos, in addition to those due to polyspermy etc., it might be claimed that only those segregations of materials that correspond with the normal location of oil, yolk, pigment etc., might give normal results. The figures of the living embryo show that the pigment and the yolk, that have practically the same distribution, and the oil may have all possible positions in the egg in relation to the axis. A study of the normal distribution of pigment and yolk shows in fact how little ground exists for supposing them to be formative materials.

The location of the visible materials of the normal eggs, and the effects of centrifuging

The eggs, en masse, have a distinct red color; the depth of color varying in different individuals. Not infrequently lots are met with that are quite colorless. The color is due to a red pigment in the egg. When seen under the microscope the egg appears faintly reddish or brownish-red (plate 1, fig. A). At one pole there is a large clear area free from pigment that is the animal pole of the egg. The opposite pole, too, is often seen to be lighter in color. Across the egg in the "animal" hemisphere there is a lighter band, (fig. A), that may be connected with the presence there of the polar spindle, that has already formed when the eggs leave the ovary.

A section of the normal egg (plate 2, fig. M) shows that the yolk lies near the surface of the egg. The yolk placques are very large compared with the size of the egg. The interior of the egg is filled with a finely granular, stainable protoplasm in which lies the large, well-developed karyokinetic spindle of the first polar body. The oil and the pigment are dissolved by the reagents and do not appear in the sections.

When the egg is centrifuged the pigment and yolk are carried to the outer pole, the oil to the inner (fig. N). Between the two lies a band of clear protoplasm. An optical section of a well-

centrifuged egg gives the appearance seen in plate 1, figs. B-F where the pigment and the oil field are flattened and sharply separated from the protoplasm.

A section of an egg centrifuged at this time is shown in plate 2, fig. N. The yolk is all collected at one pole; the pigment has been dissolved; the position of the oil is shown by the vacuolated protoplasm at the pole opposite to the yolk. The relatively large band of protoplasm contains the spindle. The spindle may lie at any angle in regard to the plane of stratification. The egg at this time does not orient as it falls in the centrifuge tube. Hence the oil and the yolk may be in any part of the egg in regard to its poles, as is shown by the formation of the polar bodies that are given off at the original pole of the egg, irrespective of the materials that have been thrown into that region as seen in figs. O, P, S.

When the egg is centrifuged at once, and then fertilized, the polar body is given off in less than half an hour. As the spindle moves to the surface it displaces there whatever material has been driven to the animal pole. A clear polar field again appears with the polar body in its middle (plate 1 fig. B- F). If the pigment and yolk are displaced, the egg appears at this time as shown in figs. B, D. The pigment (or yolk) is pushed away from the pole and assumes the form of a ring. If the spindle pushes through the oil ring, a clear region appears, fig. C, which displacing the oil forces it around the sides. If the spindle appears at the side of the clear area (figs. E, F) this region too becomes clearer than before. Under these circumstances the oil and the pigment are often carried around the opposite sides of the egg until they touch each other (fig. F). The movements of the pigment and oil fields show clearly that extensive shifting of the contents of the egg takes place at the time when the polar bodies are formed, and it is apparent that the movements are the result of materials in the interior of the egg moving up to the surface. This migration is connected with a change in position of the karyokinetic spindle.

Sections through the egg (fig. P, Q) confirm what can be observed in the living egg. I have found few cases in which the spindle moves through the middle of the oil field. This may mean that there is some mechanical difficulty in the way of such movement,

or that the oil itself slips away more easily than does the yolk, as the material wells up from the interior. The latter interpretation seems the more probable one.

The sperm may be present in the egg when centrifuged, plate 2, fig. O. It may lie in any part of the egg without regard to the centrifuging, but is displaced from the oil and yolk areas. Despite the fact that the sperm head represents a compact mass of chromatin, its weight corresponds so nearly with that of the cytoplasm of the egg that the centrifuge fails to move it. As the sperm head absorbs fluid it may be found still at any point in the egg, but as soon as the absorption passes beyond a certain point the sperm nucleus becomes lighter than the cytoplasm and is carried to the oil pole. While still small it is often found lying between yolk and cytoplasm. I have never found it embedded in the yolk. I interpret this to mean that as the yolk granules collect at the outer pole the nucleus is displaced inwards. Since it has not been found in the oil, a similar explanation may apply here.

My inability to move the sperm nucleus prevented an experiment that I had tried to carry out. Were it possible to move the sperm nucleus away from the sperm centrosome one might separate two factors, closely associated in normal development, and hope to discover whether a new centrosome would appear near the male nucleus, or whether the nucleus and aster find each other again. In the later stages of the male nucleus, when it is possible to perform the experiment, the asters are so near the center of the egg that the nucleus is within reach of their rays. I shall return to this point later.

The first polar spindle extends almost throughout the entire egg. Its displacement is more difficult, perhaps in consequence of its extension, than in larger eggs with relatively smaller spindles. Soon after the egg is laid one end of its polar spindle comes nearer to the surface. If the egg be then centrifuged one of the following results may be observed:

- (1) Should the egg fall in the machine so that the spindle lies at the side, and therefore in the clear area, it is unaffected by the centrifuging, except in so far as passage of the yolk granules may be partly blocked by the central spindle; or by the centers of the aster, so that granules may accumulate on their centripetal sides.

(2) Should the egg fall so that the spindle lies at the outer end, and comes, in consequence, to lie in the yolk, it is rarely or never displaced by the yolk. In hundreds of such cases examined I have found very few that might possibly be interpreted to mean that the spindle had been displaced by the yolk. The yolk may completely surround the outer pole of the spindle, and obliterate or obscure the astral rays, but the spindle remains nevertheless near the surface in the same way as does the second polar spindle under like circumstances.

(3) Should the egg fall so that the spindle lies at the oil pole the results are very different. The outer end of the spindle in the oil is destroyed, in part, or entirely, so far as can be judged from preserved material (plate 4, fig. 7); or the spindle is displaced more towards the center of the egg, and in some such cases the polar rays seem to be largely lost (fig. 8). Even when the spindle stands obliquely with one end at the edge of the oil cap, those of its fibres that extend into the region of the cap are lost; their loss may be due either to displacement by the oil, or to the tendency of the oil droplets to flow together into larger drops that press the fibres out of position. It is not a little surprising to find such a contrast between the effect of the oil and of the yolk on the spindle. The difference may be due in part, as just suggested, to the tendency of the oil drops to unite, while no such effect is produced in the yolk, and also in part to a greater difference in specific gravity between the oil and the spindle, than between the yolk and the spindle.

After the first polar body has been formed the inner mass of chromatin is carried over into the second polar spindle. It has not been possible to move the chromatin at any time during the transition period. The experiment should be repeated, however, on eggs in which a resting nucleus, that could, no doubt, be moved, appears at this time; for, it would be highly important to know if, under these conditions, the second polar body would be suppressed or develop at some other point of the egg, or whether the nucleus or chromatin would be drawn back to the polar regions.

The only case that I have observed of displacement of the second polar spindle is shown in plate 4, fig. 9, in which the spindle, in metaphase, has been pushed inwards from the surface. I have

seen other spindles in the metaphase that were not displaced, and also other stages in this division that showed no such displacement.

Eggs centrifuged while the second polar body is being given off offer two points of interest. The yolk is driven sometimes into the polar region, pl. 2, fig. O. The outer end of the spindle is obscured by the yolk; at best it is not well developed when the spindle is near the surface. It will be noted that the yolk is driven into the region all around the spindle but not within the spindle itself. In other words the materials that surround the spindle are permeable to the yolk spheres; at least when as in this case the spindle is in a late phase of division. It is probable that the egg weighted by the polar body tends often to orient as it falls: at least I have rarely found the oil cap in the polar field but this may be due to the polar spindle becoming displaced by the oil.

The egg quickly reaches the stage where two pronuclei are present. At their fullest development these nuclei are very large compared with the rest of the egg, plate 4, fig. 10, and lie near its middle. During these stages the two pronuclei are easily moved by means of the centrifuge. As shown in plate 2, in fig. R, and plate 4, fig. 11, 12; plate 5, 13, 17, 24; they are carried beneath the oil cap, touching it on their outer ends, but not penetrating it. The specific gravity of the pronuclei is obvious from their position. They are heavier than the oil, lighter than the yolk, and lighter even than the cytoplasm.

A large number of experiments were made at this stage of development in order to study the problem of the formation of the segmentation spindle when the nuclei are carried into foreign parts of the egg. While nuclei in the resting stage may be moved, my experience has been that in *Cumingia* asters and spindles can rarely be moved unless the protoplasm about them is shifted bodily. It follows that the centrosome (and its aster) brought in by the sperm will be left where it lies when the nucleus is removed, unless the two are attached by the rays of the aster. In the latter case it is possible that the nucleus would be swung around and carried to the oil cap drawing the aster after it through the egg. Something like this appears to happen, although the point is difficult to settle positively, owing to the different possible posi-

tions of the aster and nuclei before centrifuging with reference to the direction of centrifuging. I have not studied in detail the normal fertilization in *Cumingia*, but there is every evidence that the egg follows the typical process of the group to which it belongs, viz., a centrosome and aster appear near the male nucleus, divide, and produce the segmentation spindle, plate 4, fig. 10, that collects the chromosome from both the male and female pronuclei. The spindle takes such a position in the egg that the first plane of cleavage passing through it also passes through the pole of the egg.

If the egg has been centrifuged during the earlier pronuclear stages I often fail to discover the aster either because of difficulty in locating it or because it is obscured to some extent by the centrifuging, or because the series of sections is imperfect; but there is no doubt that it is present; first, because a spindle appears in each egg at the same time as in the normal, and secondly, because in polyspermic eggs as many asters (or pairs of asters) appear as there are male pronuclei present. This latter point is a matter of no little importance. It shows that the centrosome and aster are actual things that exist independently of the nuclei that come with them into the egg; for, in this case the nuclei have been carried away from the asters by the centrifuging and yet the asters continue to go through their regular phases. No new asters develop near the nuclei in their new position—at least I can find no evidence of such neo-formation—but in polyspermic eggs a cluster of asters appears beneath the nuclei, plate 5, fig. 15. The evidence is a virtual demonstration of the independence of the asters.

In later pronuclear stages the asters are large and are nearly always found near the center of the egg beneath the pronuclei, plate 4, fig. 12; plate 5, figs. 16, 18, 23, 24. Fibers from the aster run to the pronuclei where they seem to be attached. This relation suggests that as the nuclei are carried toward the oil cap they often still remain anchored by one side to the old aster. If this is the correct interpretation the nuclei must revolve as they move.

We come now to an important question—how do the spindle and chromatin in these eggs with displaced pronuclei get into

position for the first cleavage? Do the nuclei move back independently of the spindle? Does the spindle first form near the nuclei and then move into position? Sections give a clear answer to these questions. Eggs were killed and preserved at close intervals after the nuclei had been driven into the excentric position. Sections of some of these eggs are shown in plate 5, figs. 14-22. It is clear from their figures that the nuclei do not move back to the center of the egg but resolve themselves in place; even before they break down, the chromatin begins to move towards that side of the cell nearest the aster. The fibres of the aster are attached at this point. There is no evidence that they penetrate as yet the nuclear field, but there is abundant evidence that the chromatin in some way draws to the asters and later enters the spindle. In the early stages of my work I had obtained some eggs in which the chromosomes seem to be drawn out from the centers towards the oil cap as in the egg in plate 4, fig. 11, and this led me to think that they had been moved. It is now clear that the resting nuclei had been moved and the chromosomes were drawing over towards the asters.

In another and better way I studied the same phase of development. When two pronuclei were present the eggs were put on a water centrifuge and kept there until the segmentation had begun in some cases. Were it possible for the pronuclei to move back when the eggs were taken from the centrifuge, it would not be possible for them to do so, I argued, if kept all the time on the machine. In fact the nuclei remained in their forced position. Nevertheless a spindle formed and cleavage took place, the cleavage plane passing through the animal pole of the egg. Eggs treated in this way have been extensively studied, and in fact the figures of plate 5, 13-22 are from these eggs. The movement of the chromatin towards the aster is clearly seen in figs. 16, 17, 18. The movement of the spindle into position after it has accumulated its chromatin is also evident in many of the sections. The spindle may move towards any point of the egg without reference to the presence there of oil or yolk. It appears to assume its normal position in regard to the polarity of the egg. The spindle moves despite the fact that it is on the centrifuge during this time. It is

able, indeed, to move out of its path either the yolk or the oil if they stand in the way of its assumption of its proper position. If we knew the relative weight of the different materials of the egg and the rate of centrifuging that just permits their removal by the spindle, we might I should think calculate the forces that bring the spindle into position.

The behavior of the chromatin at the time of its transfer from the nucleus in forced position to the spindle, calls for special treatment. In many of the nuclei the chromosomes appear bunched together in masses of varying size (figs. 17-20). These masses may pass in this condition on to the spindle. Their subsequent history is difficult to make out. It appears that in most cases the chromosomes separate as they pass into the equator of the spindle. Presumably they then divide, but whether sister chromosomes always pass to opposite poles, or whether they may sometimes be unequally distributed I do not know.

The question arises as to what causes the heaping up of the chromosomes. There is no evidence that it is caused by the centrifuge and the evidence is very strongly in favor of the view that the chromatin in some way draws towards the aster pole. The most probable interpretation is, I think, that these nuclei lose their orientation to the aster at a critical time and are unable to regain it before the chromatin is resolved into its chromosomes. The irregular position of the chromosomes with reference to the aster is the cause of their heaping together which is avoided in the normal egg by the pole of the nucleus being oriented to the aster which permits the ends or angles of the chromosomes to pass directly without interference to the spindle.

Whatever be the cause of the heaping of the chromosomes, one important fact emerges, viz.: the chromatin material is moved or is drawn as a mass to the aster that is nearest to it. The bearing of this evidence on the interpretation of movement in the chromosomes will be discussed later.

In eggs that have been kept on the centrifuge from the time of formation of the pronuclei until the first cleavage is completed, I have noticed that the nuclei in the blastomeres are often extremely small (plate 6, fig. 28 and plate 2, fig. U). Compared with

the normal nuclei (plate 2, fig. T) these seem out of all proportion. Also during the construction phase of these nuclei the chromatin-mass often appears condensed. It is difficult to explain this result unless it means that some constituent of the nucleus is lost in the centrifuged egg or at least that less of it passes on to the spindle. Possibly the conditions shown in fig. 31 may account for the result. This egg was centrifuged as the two nuclei re-formed. The watery fluid that collects at this time is carried to the oil cap. Its loss may account for the reduced size of the nuclei.

In a few eggs, that were centrifuged when the spindle was well developed, I have noticed a remarkable condition in the center of the aster. As shown in plate 2, fig. S, a yellow ring, that stains like yolk, lies around a central granule. Whether in reality one or more yolk granules have passed into the more fluid (?) center of the aster and been caught there, or whether we are dealing with an artefact, I can not state. But the result is so peculiar that it seemed worth while to draw attention to it.

The movements of the materials that take place during the first cleavage can be studied both in the living egg and in sections. When the cleavage passes through the oil pole the oil is drawn down along the plane of division as far as the former center of the egg. Similarly for the yolk as seen in sections in plate 2, fig. U. This egg divided while on the centrifuge. Doubtless the forces that produce division sufficed to carry in the yolk against the centrifugal force acting in the opposite direction.

If the eggs are allowed to complete the first division before they are centrifuged, each cell is stratified into three layers, plate 6, fig. 27. The nuclei are driven to the regions beneath the oil mass. Nevertheless at the next division the cells divide typically irrespective of the distribution of the centrifuged materials. The result means that despite the forced position of the nuclei the spindle develops in its normal position, and gets its chromatin from the nucleus in the same way as does the segmentation spindle.

If the egg is revolved in the four-cell stage, again the individual cells are centrifuged, plate 2, fig. V, and the same holds for the 8 and 16-cell stages. Later stages than these I have not tested.

The interest in the experiment is twofold; it shows to what extent the yolk, oil and pigment are distributed to each cell in development, and it shows, or might show, how soon these substances are used up in the respective cells or whether they are added to during development. In *Cumingia* it is evident that all the early cells get yolk, oil, and pigment, the relative amount being in proportion to the size of the cells. The distribution of the pigment and yolk, plate 2, fig. M, is such as to lead directly to such a result, provided they are not segregated during development, which does not appear to be the case from the evidence here furnished. The oil cannot be readily identified in the normal egg, but obviously it, too, must be equally diffused in the egg. There is no evidence that any of these substances increase during the early cleavages, or that they are used up, at least in amounts that could be detected.

The evidence shows, therefore, that while these three visible substances, yolk, oil, pigment, are present in all the early blastomeres, their absence from these cells does not prevent normal cleavage or development and their presence in excess does not interfere with the normal process.

Centrifuging eggs in the ovary

In the vain hope that the chromosomes might be moved at the time when the large germinal vesicle breaks down, I tried to obtain eggs in this condition. The *Cumingias* were put into dishes of water and just before the expected time of oviposition, or just after the first eggs were set free, the animals were opened, the ovary taken out and the pieces centrifuged at once in the tubes. In most cases the germinal vesicle had disappeared, even while the eggs were still in the ovary; or had not broken down at all. In only two or three cases did I obtain eggs in an intermediate condition. In none of these were there evidences of the independent movement of the chromosomes. The spindle that is already present in many of the eggs, although feebly developed, was in connection with the chromosomes, plate 4, fig. 4.

The eggs while still enclosed in the ovary show the polar spindle; and in some cases I have found sperm nuclei in the eggs, showing that if sperm are in the water they may penetrate to the ovary and fertilize the egg there. But fertilization is not essential to the ripening of the egg, as shown in cases where the eggs are laid in the absence of the male.

The effect of centrifuging the ovarian egg is shown in plate 4, figs. 1-6. When the germinal vesicle is intact it is carried to one side of the cell; above it an oil cap forms, but the cap is less apparent than in eggs that have been laid, and also less concentrated, figs. 1 and 2. It is in fact sometimes difficult to detect the oil cap in the ovarian eggs. The yolk also appears less easily moved. At first I was inclined to interpret this as due to the more fluid parts of the egg being still retained in the nucleus, but the same conditions, although less pronounced, are present in ovarian eggs in which the germinal vesicle has been dissolved, figs. 3-5. It appears, therefore, that the egg must absorb water after it is laid, and becomes, in consequence, less viscid: so that its lighter and heavier substances are more easily separated.

General considerations

Four points of interest are particularly well illustrated by the action of a centrifugal force on the egg of *Cumingia*.

First: The question of the rôle of the visible substances of the egg as organ-forming materials;

Second: The formation of the aster and its relation to the chromosomes;

Third: The movements of the aster in the egg;

Fourth: The bearing of the results on the phenomenon of cleavage.

These points may now be discussed in turn, and although the last three are more closely related to each other than to the first, yet their correct interpretation has an important bearing on the first question.

The rôle of the visible substances in the egg as organ-forming materials. The results with the egg of *Cumingia*, an egg that shows

all the characteristic features of precocious development, tally completely with the results on the frog and sea-urchin. The visible substances of the egg that can be centrifuged are not organ-forming. Centrifuging may, it is true, cause abnormalities in the development in more than one way, but such abnormalities can generally now be referred to the real cause and that cause is not the segregation of the visible materials of the egg. It has been well worth the time and trouble that it has cost to work out these other causes of abnormalities; for, there has been a tendency on the part of some embryologists to attribute all abnormal development after centrifuging to the lack of redistribution of the visible materials. The repeated failures in the case of *Cumingia* to get normal embryos after centrifuging well illustrate this point. By a fortunate circumstance the small size of the animal made it possible to centrifuge the eggs within the animal and to have these eggs laid normally, when normal development was the rule.

Some other causes of abnormal development may be briefly referred to. In eggs with a large amount of yolk its transportation to one part of the egg may interfere with normal development, because of the mechanical difficulty of nucleolization of this part of the egg, or because of difficulties in the movements of yolk-laden cells during gastrulation. The compactness of the yolk-mass may likewise interfere with the orderly sequence of the cleavage, etc. In eggs kept in the centrifuge for a long time the resting nuclei may be carried so far away from the spindle that their chromatin may fail to reach the spindle, or else reaches it in such a form that an irregular distribution of the chromatin takes place with injurious after-effects. Even in an egg as small as that of *Cumingia* such effects are visible.

Handling the eggs may affect them in various ways, as is excellently shown in the present case. The sojourn of the eggs in a confined space may also seriously affect them, if kept there for a long time.

These and many other factors may induce abnormal development. Despite these possibilities the results show plainly that normal development may follow when the visible substances

are unequally distributed, and are carried over into the blastomeres, redistribution being thereby prevented.

The formation of the aster and its relation to the chromosomes. The experiments in which the sperm-nucleus was carried away from its aster and only later was brought into relation with the segmentation spindle show that the sperm aster develops independently of the nucleus. In other words the aster does not appear to be a manifestation in the cytoplasm caused by the vicinity of the nucleus, but to be an independent development. The case of centrifuged polyspermic eggs also illustrates the same point. The evidence from a study of normal fertilization has shown that the aster develops around some body brought in by the sperm, also the experimental evidence makes clear that the material, so brought in, acts as the future center for aster formation. The genetic continuity of the centrosome thus introduced can in many cases be demonstrated, and even when it cannot be, the evidence still makes probable the view that new centers form in connection with the old. This problem of the continuity of the centrosome and aster is, however, one that has not lent itself to such diagrammatic treatment as in the case of the chromosomes. Several years ago when Boveri proposed the theory that the centrosome was a body introduced by the sperm as the essential feature of division, I showed that by treating eggs of several animals with salt solution, artificial asters appear. A study of their origin revealed that they were not derived from a single center—as might have been claimed since the egg itself had also a past connection with centrosomes—but appeared independently at any points in the egg. Their exact method of formation I was able to make out for several kinds of eggs. These asters resembled in every way the normal asters, including a small deeply staining center or centrosome. They waxed in size as the cleavage process approached and faded later. They attracted the chromosomes as do normal asters. Wilson demonstrated later that they divided, and produced a spindle between the separating centers.

It is clear that while in normal cell division a continuity of the centrosomes may exist, that insures the regular occurrence of

cytasters, yet the formation of these bodies may also take place independently of the original centers. These views are not, I think, incompatible but rather supplementary. If we look upon the centrosome not in the light of a cell organ, but rather as a center about which the ray formation is more likely to develop than elsewhere, we can harmonize many kinds of evidence. When I think of the phenomenon as a process similar to crystallization I get so much clearer a conception of it that I am tempted to describe it in this way. When, for example, the egg of the sea-urchin is put into concentrated sea water numerous stars are slowly formed. The center of each star would be formed wherever the concentration began to be a little denser than elsewhere. About these centers the crystal rays would develop. The centers might remain, *in potentia*, when at a later phase of the cell division the crystallizing forces had weakened, owing, let us say, to the absorption of water, for which there is some direct evidence. The center might again act as the central points in the formation of the next asters.

From this point of view the spermatozoön brings into the egg a crystallization center about which the ray formation would take place more readily than elsewhere. The rays would represent real, although temporary structures. Their formation would cause certain changes in the equilibrium of the cell that would lead finally to the division of the cell about each aster as a center. We might think of such systems developing in certain directions more easily than in others; these directions corresponding to a stereometrical condition of the egg plasm. Cytologists have often discussed the question whether the rays represent lines of force in the sense that the aster is a dynamic center controlling the changes that take place about it. On the view here suggested the center is not a center of emanations or diffusion, for, it does not exert an action at a distance through the cell, but is a center about which a system is built up depending on the crystallization properties of the molecules of the protoplasm. The rays are lines of force only in the sense that they represent the forces of crystallization. The rays are not lines of force in the sense that the center is exerting a radial influence on the cell or that the center itself is a dy-

namic force. My interpretation is more in harmony with what we see, and is more in accord with the well known changes that take place as the asters form and reform in the egg at its different phases. A further consideration of this question may be deferred until the experiments with *Cerebratulus* have been described.

I have given the evidence that seems to me to show that the chromosomes move or are drawn towards the asters to whose fibers they appear to become attached. It has long been debated whether the fibers draw the chromosomes to the center, as an elastic fiber might be supposed to act, or whether the chromatin glides along the fibers as paths. Concerning the mechanism of the process I have nothing new to offer, but I see no difficulty in bringing the movement into line with the suggestion offered above that the fibers of the asters are crystalline products of the cytoplasm, for however they act they represent lines in the cell that differ physically from the rest of the cytoplasm, and the chromosomes might react to such lines in a way different from the way in which they react to the rest of the cytoplasm.

The movements of the aster in the egg. The migration of the asters through the cytoplasm towards certain well defined regions and the movements of the spindle as a whole are well known phenomena to every embryologist. The mechanism of these changes is entirely unknown, but we know that the movements follow certain axial relations of the egg. In cleavage it is known that the asters behave in a perfectly definite manner, that is connected with the cleavage pattern and, presumably, with some system in the egg. Their migration can be readily affected by pressure applied to the egg, and from this evidence, I have argued elsewhere, that it seems probable that the rays develop in accordance with the lines of tension in the egg. It has been generally taken for granted, I think, that the movement of the polar spindle to the animal pole of the egg is in some way connected with the presence in that part of the egg of materials that make it easier for the spindle to move to this point, or else, it has been assumed, that the spindle takes the shortest course to the surface irrespective of what point of the egg surface lies nearest to it. Both of these views are refuted by the evidence from the centrifuged egg. It has been shown that

the polar spindle moves to the pole of the egg irrespective of the materials that have been driven into that region. The spindle will push through the dense mass of yolk if that lies in its path, or push the oil cap to one side should it lie in the polar regions. The placing of the segmentation spindle shows again that the spindle orients with respect to the poles, however much the nuclei are driven out of position.

These facts lead irresistably to the view that the position assumed by the spindle is not hap-hazard, but in direct response to conditions existing in the egg, that are independent of its visible materials. This goes far towards showing that the egg is something more than a mixture of fluids, and that a system exists in the egg of such a kind that it has a guiding influence on the formation and movement of the karyokinetic process. That this system is not rigid, but flexible, is also shown by many experimental results. We shall have to include, I believe, in the physiology of the egg the recognition of directive factors of a physical nature. The physiology of form-changes will have to reckon not simply with physiological changes in enzymes or stuffs, but with physical factors as well.

The bearing of the results on the phenomenon of cleavage. The most important fact brought out by the study of *Cumingia* is in regard to the nature of the cleavage process. It has been shown that in the first division, which is very unequal, the smaller cell may contain all of the oil, or all of the yolk, or pigment without thereby changing the size of the cell or its relation to the pole. Sections show that the small cell may be almost entirely filled by the yolk or by the oil, plate 1, figs. G, H, I and plate 6, figs. 29, 30. This means that the greater part of the cytoplasm in these regions has been almost totally displaced, yet the normal size ratio of the cells is retained. How can we explain such a result on the ground that the form of the cleavage pattern is determined by regional differences in the egg. It is true that the yolk and the oil do not totally displace the cytoplasm, but are permeated by it. But the quantity must be often very small compared to that of the introduced materials, and if the different regions are characterized by differences in the cytoplasm the proportion of the cells would have

to be very greatly altered to include the cytoplasm of these regions in addition to the introduced substances.

The evidence seems to me well-nigh conclusive that the size of the cells is not determined by regional differences but by the karyokinetic spindle, and its surrounding materials. It has been shown that when a spindle moves into the yolk or oil it displaces these materials to a certain extent. In such cases it may appear that the materials introduced from the interior of the egg with the spindle are responsible for the proportionate sizes of the cells. But the amount introduced is small compared with the cell as a whole which may still contain a large amount of oil or yolk, so much so in fact that in the living egg it appears to be entirely filled at times with one or the other of these materials. The question that arises here may, however, be met by those cases in which the egg is centrifuged after the segmentation spindle has been formed. In consequence the yolk or oil may be closely aggregated around the outer pole of the spindle, yet the division that follows is into two unequal cells of proportionate size.

It should be recalled that the karyokinetic figure occupies practically the whole cell. By driving oil or yolk to one end of the spindle the fibre system is left largely intact. It is this system that appears to be the determining factor in the division, and not the materials that fill the rest of the cell. On this view, and this alone, can we understand how proportionate cleavage takes place under the conditions introduced by the centrifuge. This conclusion brings into the foreground, I believe, the important rôle that the karyokinetic figure plays in division, and conversely directs attention away from the chromatin, the nuclei, and the remainder of the cytoplasm that does not take part in the spindle formation. The conclusion shows that theories of cell-division that assume the nucleus to be the controlling factor, or its chromatin, and the theories that rest on cytoplasmic regional differences apart from the spindle may have ignored the essential factor in cell division—the karyokinetic figure.

2. CENTRIFUGING THE EGG OF CEREBRATULUS

The eggs of *Cerebratulus* are large and laden with yolk. They can be obtained at the proper season in large numbers. The maturation takes place in the course of one to two hours after removal affording a good opportunity for applying a centrifugal force during this entire period. Since these stages could not be advantageously studied in *Cumingia* I was anxious to make use of a form like this, whose eggs mature after removal. In another respect also *Cerebratulus* differs from *Cumingia*. If strongly centrifuged the eggs may be drawn out into bottle-shaped forms (plate 7, figs. 37, 44) and the neck of the bottle even separated from the body (fig. 36, 38). The effect of the change in shape on the karyokinetic figure offers certain points of interest.

Through the courtesy of Professor W. R. Coe of Yale University I obtained in his laboratory an abundance of material during March, 1908. At the Harpswell Laboratory I obtained further material during the summer of 1909. The combined results are here given.

The enormous germinal vesicle lies slightly eccentric. Between it and the pole of the egg the cytoplasm contains less yolk and through this part the polar spindle reaches the surface. The cytoplasm is filled with large yolk granules and these are more abundant towards the antipole. When the egg is centrifuged, slowly a strange effect is sometimes produced as shown in plate 2, figs. W, X. A ring of yolk granules lies below the nucleus. Its interior is filled with a less stainable material with yolk granules scattered in it. Outside of the ring a purplish zone is found whose color is due to the mixture of yellow yolk and blue cytoplasm. If the egg is rotated for a longer time, the ring becomes solid, and finally the resulting ball of yolk is thrown down to the bottom of the egg (plate 7, fig. 37). On that surface of the ball that is turned towards the nucleus a zone of black-staining, finely granular substance can be distinguished, and these granules are also found throughout the ring especially on its outer and inner surface, (plate 2, fig. X).

The rest of the egg is filled with cytoplasm having a granular

appearance in the preserved egg. A broad cap corresponding to the oil cap of other eggs is found between the surface of the eggs and the outermost wall of the displaced nucleus. Since the material is not dissolved out in the alcohol, xylol or acids, it is probably not oily. Within it however a few vacuoles may often be found which probably are the holes left by dissolved oil. The scattered granules, mentioned above, that stain with basic dyes, are also present and are driven with the yolk to the outer pole. They form the dark cap sometimes present on the yolk sphere. The formation of the yolk ring is difficult to explain. I have not found it conspicuous in the eggs obtained at Harpswell. Differences in the rate of centrifuging in the two cases are no doubt responsible for the results. The New Haven eggs were probably rotated more slowly; but the difference between the two sets cannot be entirely explained by the time of centrifuging but perhaps by the preservatives, since Harpswell eggs, rotated only fifty times, show no evidence of rings although the yolk has begun to move to one pole.

The relation between the yolk ring and the nucleus is interesting. As the ring moves from the periphery of the egg towards the center of the egg it encloses in its polar portion the germinal vesicle. A later stage shows the nucleus passing through the ring as the nucleus moves to the pole and the ring towards the antipole.

Obviously the material of the ring is made up of the yolk granules situated near the surface of the egg, but this does not explain why the yolk granules move inwards towards the center of the egg; rather should we expect them to move by the most direct path to the antipole, unless the interior of the egg is more fluid and therefore less resistant to the yolk granules than the more cortical layers. Lillie explains the similar ring formation seen in *Chaetopterus* in this way, and I incline to adopt his suggestion. The yolk spheres move with low centrifuging in an oblique course outwards, in consequence of the centrifugal force, but somewhat towards the center of the egg because the resistance is less here than in the cortical layer. When a high rate of centrifuging is employed the yolk granules respond more directly to the centrifugal force.

Orientation of the eggs in the centrifuge

When the eggs are centrifuged before the germinal vesicle breaks down, the latter is driven to one pole; if centrifuged not too hard, the yolk ring forms; if hard, the yolk passes more directly to the outer hemisphere. If these eggs are kept until the time when the polar spindle forms and then killed and preserved it will be found that the polar spindle reaches the surface in position to give off the polar body only in the lighter hemisphere, never in the yolk hemisphere. This may mean either that the eggs orient so that the pole is towards the center and the antipole outward; or that only those spindles can reach the surface that are in an egg whose pole is in the lighter hemisphere; while those in eggs with their pole in the yolk hemisphere fail to penetrate the yolk. The second conclusion is, I believe, the correct one. In support of this view is the fact that in many of the eggs polar spindles can be found at this time in the interior of the egg, and some of these contain the chromatin in the form of two separate plates near each pole of the spindle. Such a condition is found in the normal spindle only when the polar body is ready to be given off. Lillie has found a similar condition in *Chaetopterus* and interprets it in the same way.

If the eggs are centrifuged after the polar spindle is formed the same state of affairs is found; spindles are found in the light hemisphere at the surface and inside the egg; none in the yolk hemisphere. In this case those spindles that lay in the yolk hemisphere must have been driven into the interior of the egg.

Eggs that have given off the polar bodies have been examined, but so far without much success, since it has been difficult to detect the polar body with certainty after centrifuging, but the evidence so far as it goes indicates that polar bodies may lie at any point on the surface of the egg, and this condition is opposed to the idea of orientation on the machine.

In *Cerebratulus* there is, for a time, a long or short stalk of protoplasm at the antipole of the egg. I have tried to determine the method of falling of the egg by this means. Since the stalk lies within the jelly membrane it probably has no directive influ-

ence on the falling egg, and its weight would seem too insignificant to act as a load. So far as the evidence goes, it shows that eggs fall at random, but the stalk is lost in many eggs, making identification difficult. It should, of course, not be overlooked that a partial orientation of the egg may be possible. Those eggs that have a long distance to fall may orient, while those already at the bottom of the tube may become caught by surrounding eggs and fail to orient. Only a proportionate count of eggs in relation to the location of the polar spindle could settle the question.

The evidence on the whole is in favor of random falling for the egg of *Cerebratulus*, *i.e.*, the eggs do not orient, at least not to any considerable degree.

There is a consequence of some importance involved in this result which shows that the distal spindles are carried centralwards. This means that the spindle retains its form although carried half way through the egg in consequence of the rearrangements involved. If the centrosomes be centers of force we must suppose that in their passage through the egg as just described the asters are in process of reformation and absorption at every step of their progress. This is as necessary to the center of force assumption as it is necessary to assume that particles of iron rearrange themselves as the magnetic poles move through them. But when we recognize the time element in the building of the aster in the normal egg it seems incredible that this interpretation can be correct. Possibly the situation might be met by the assumption, that, not only the spindle, but its surrounding medium, is moved as the yolk fills up the lower hemisphere. Opposed to such a view is the fact that the yolk does not completely fill its hemisphere, but lies imbedded in the ground material already present there, and this material is sufficient in amount to bring about division, showing that it is not inconsiderable. Moreover in some eggs the polar spindle passes through the yolk to reach the surface, showing that there still remains in the yolk field enough of its material to act as a polar field. But it may not be so much the amount of material as the molecular directions of that material that give the axial relations.

The evidence, while it does not disprove completely the center

of force conception, still puts that assumption on rather a questionable basis.

Lillie has recently pointed out that he has found a decisive proof of the center of force hypothesis in the behavior of the spindle when the basic granules of the egg are driven into its field of action. These granules now become arranged in chains that represent the polar rays and replace the original material of these rays. If Lillie's proof is cogent, it is the most important fact yet discovered, by means of the centrifuge, in relation to the process of karyokinesis. Therefore I propose to examine it as critically as possible, since it involves our entire conception of the meaning of the karyokinetic figure. The egg of *Cerebratulus* furnishes the same kind of evidence as that adduced by Lillie, so that I am able to consider his conclusion from the evidence of my own observations.

It is true, as Lillie has found, that when the rays from the poles of the spindle pass into a region rich in basic granules these granules appear to be arranged bead-like. From this fact Lillie argues that the beads have taken the place of the fibres under the influence of the outpouring of the centrosomes or centers of some sort. But it should be remembered that in all normal cells containing such granules these become arranged along the lines of the spindle fibres as the fibres pass by them. Possibly when the alveoli are narrow the granules may become imbedded in the fibres. This point is too difficult to determine with certainty. The granules appear to lie in the walls of the larger alveoli and it is through these walls that the astral fibres pass. It is not surprising, therefore, to find the granules arranged along the fibres to which they appear to be stuck.

If these granules are sufficiently numerous they may obscure the less stainable fibres, and hence lead one to conclude that the granules have replaced the fibres. It seems to me that Lillie may have fallen into this error but whether he has or has not, the evidence that he adduces is of such a kind that I venture to question the certainty of the interpretation. I do not wish to speak dogmatically on the point, for I realize how difficult it is to be sure that fibres cause the orientation of the granules and that the granules take no part or play only a secondary rôle in the process.

But this very difficulty of proof is evidence of the insufficiency of the demonstration derived from this source.

The best evidence that I have to bring forward against the center of force hypothesis is derived from the spiral asters.

When the eggs of *Cerebratulus* are drawn out into strings the polar asters or the sperm asters or the asters that will form later the poles of the spindle are carried out into the elongated mass (plate 7, figs. 36-45, and plate 8, figs. 47-54).

In some cases these fibres are destroyed especially at the outer ends, but in most cases they are present and retain their radial structure, as shown in fig. 36. This result would seem to mean that the fibres of the young asters are relatively rigid, and not easily disturbed by the flow of protoplasm about them. But the same facts might be interpreted to mean that the centers of force that are drawn out in the flowing mass reform the asters wherever they move.

However if the eggs are centrifuged when the large karyokinetic figure of the segmentation phase is present the result of the centrifuging is shown by the asters. These are drawn out as the spindle or the reconstructed nuclei are carried towards the pole of the egg and bent, at many different angles as shown in plate 6, in figs. 32-33 and plate 8, figs. 47-53. When the course of one of the poles has been oblique, the fibres are thrown into beautiful spiral forms as shown in figs. 48, 50. The results give every evidence that the fibres are actual fibres that have a real existence in the eggs. Their existence may be temporary, but while they last they appear to be denser portions of the network. On such an assumption the bent course of the fibres here described can be explained. On the center of force hypothesis these conditions are inexplicable.

The fate of the inclosed polar spindle

In the series of preparations at my disposal I have not been able to follow the fate of those eggs in which the polar spindles, failing to rise to the surface, divide within the interior of the egg. Many problems of peculiar interest are connected with their history. Whether such eggs develop parthenogenetically owing

to the retention of all the chromatin; whether they become fertilized and if so, whether they produce normal embryos; whether the polar centrosomes disappear if replaced by the sperm aster, and, if not, what their fate may be; are all questions of prime importance.

There is no evidence that these eggs develop by parthenogenesis. This suggests that parthenogenesis may have nothing whatsoever to do with the number of chromosomes in the egg. Hence those theories that try to explain the extrusion of the chromatin as a process to prevent self-development may be discarded, as well as those that explain fertilization as the outcome of the addition of more chromatin to the egg.

The failure of the egg to divide at the time when the chromosomes move to the poles of the polar spindle seems to be due to the smallness of these spindles at this time, and since delay does not increase their size, their size must be determined by conditions existing in the egg prior to extrusion of the polar bodies—conditions that are subsequently set aside by the introduction of the aster from the male. The small spindles of the polar bodies suffice, however, to bring about division of the egg when carried to the surface. This result supports the view that I have suggested as to the function of the spindle.

In some of the centrifuged eggs that correspond to the controls with two pronuclei, I have found a triaster in the middle of the egg. The triaster concerns only the polar spindle and is not due to the addition of the sperm aster as shown by the small number of chromosomes, (plate 8, fig. 57.) The most probable interpretation is that one pole of the imprisoned spindle has divided and the chromosomes—whether divided as yet or not is not clear—have moved in part into the new spindle. This division of one pole may correspond to the division of that pole of the polar spindle that remains in the egg after the first polar body has been formed. The interpretation suggests that there is a difference between the poles of the polar spindle and that it is not fortuitous which one turns outwards towards the surface.

In some eggs, which, as to time, correspond to the segmentation stage I have found spindles like those shown in fig. 58. There is a

large barrel-shaped central spindle containing a large number of chromosomes and fibres, and two polar asters with few fibres resembling in every respect the asters of the polar bodies. The fibres that run from these to the central spindle are far less numerous than the fibres of the spindle and the poles appear quite reduced compared with the large poles of the segmentation nucleus. Provisionally, I interpret the result to mean that the chromosomes of the polar spindle have divided once or twice, but the polar centers have not divided. The chromosomes or the nucleus from which they are derived are responsible for the central spindle. The number of the fibres of this central spindle is determined by the chromosomes or whatever goes along with them in the nucleus. Their number corresponds with that of the chromosomes. The asters, however, are those belonging to the polar body; the number of their rays now falls far short of those of the central spindle, hence the peculiar type of karyokinetic figure that is found.

Should this interpretation be established it suggests that the fibres of the central spindle of the karyokinetic division arise from nuclear material, or possibly from the chromosomes themselves or from the linin; and that the rays from the centrosomes establish a secondary connection with these. I hope by further observations, more completely controlled, to test the validity of these inferences.

Segmentation of the centrifuged eggs

Segmentation has been studied in eggs centrifuged at various stages prior to division. In general it may be said that the yolk sphere does not break up and redistribute the yolk granules prior to division except perhaps to a limited degree. The yolk mass appears to offer a serious obstacle to the passage of the cleavage furrow as shown in plate 6, fig. 35. The development of such eggs has not been studied in detail. In some cases where the yolk is not too compact the cell wall cuts through and equal cells result. From these the normal embryos that have been found in some cases probably develop. In many of the first segmentation

stages the animal halves of the blastomeres separate widely and a clear fluid appears along the line of division. How far these effects are artificial I cannot state since comparative studies with differing reagents have not been made. Unfortunately most of the Harpswell eggs were killed in picro-acetic, which while giving good figures of the asters and spindles does not appear to preserve the cytoplasm particularly well, forming a coarse reticulum. Eggs preserved in corrosive acetic are much more finely granular and appear in other respects better preserved.

In many eggs the first division is at right angles or oblique to the plane of stratification, cutting off a cell free from yolk and another containing all the yolk. These eggs are those in which the centrifuged axis did not coincide with the polar axis. They were not isolated unfortunately to see whether the presence of the yolk ball would cause abnormalities in development, but it seems probable that this would be the case; for, the compact yolk seems to interfere seriously with the segmentation.

Unless the eggs are in excellent condition normal embryos do not develop. It is also necessary to keep the eggs under favorable conditions, free from slime and sperm, to obtain the characteristic larvae, the pilidia. My studies were confined largely to the immediate effects of the centrifuging on the eggs and insufficient care was given to the embryos. In some lots of centrifuged eggs I have, however, obtained as high a percentage of pilidia as in control sets, but the observations do not suffice to show more than that some eggs develop normally. There can be little doubt that the yolk ball introduces abnormalities, but how far I cannot state.

Influence of centrifuging on the karyokinetic figure

The eggs of *Cerebratulus* are particularly well suited to study the influence of centrifuging in the various phases of karyokinesis and the evidence throws some light, I believe, on the nature of the asters and spindles.

If centrifuged at once and allowed to stand the polar spindle forms near the inner wall of the nucleus. As the spindle develops the chromosomes are drawn or move to its equator. The rest

of the nucleus changes its staining character and becomes incorporated in the general cytoplasm. Its extensive net-work and fluid are set free, and the rather small chromosomes collect in a mass near the spindle. One realizes how small a part of the germinal vesicle goes onto the spindle, and how large a part is contributed to the cytoplasm. Centrifuging the egg during the period of dissolution fails to move the chromosomes away from the spindle. In some eggs clear lumps appear in the cytoplasm, derived from the nuclear sap but it seems likely that these eggs were immature, for, these clear regions do not appear in the best sets.

The polar spindle lies at first deep in the egg. If at this time the egg is centrifuged very hard the cytoplasm may be drawn out to produce the bottle-shaped figures (plate 7, figs. 36, 37, 44) mentioned above, or even, string-like processes. The latter representing the yolk-free parts are often broken off from the yolk mass figs. 41-43. The polar spindle may come to lie in the "neck," fig. 37 or even in the isolated fragment, fig. 38. Despite the great changes in the cell body, the spindle and the asters at its poles retain their characteristic shape. The rigidity of the fibres is admirably demonstrated under these conditions. The fibres are rarely displaced, or bent, or broken despite the extensive movements that must take place in the surrounding plasma, yet sometimes they are lost.

I hoped to be able to determine whether fragments of the egg containing the polar spindle set free polar bodies, but the preparations do not furnish sufficient evidence on this point.

Eggs centrifuged during the period of extrusion of the first and second polar-body have shown that the spindle and chromosomes move as a whole, and cannot be separated, and are seldom moved except when replaced bodily by the yolk. On the other hand when the pronuclei are formed these are readily transported to the polar cap. In this regard the results are the same as in *Cumingia*. Preparations of eggs killed at this time show that the male aster (or asters) is also as a rule carried with the nucleus to the pole, and is found attached to its more centrally lying side, plate 8, figs. 47, 48, 49, 52. Despite its enforced passage through

the cytoplasm, the aster retains its form showing the relative rigidity at this time. Incidentally the results indicate as pointed out, that the movement of the aster in the egg may be purely passive, and not be due, as has been suggested, to its re-formation at each step in its process.

Eggs centrifuged during the pronuclear period, and returned to sea water, show a spindle near the center of the egg that resembles the normal segmentation spindle. In this case as in *Cumingia* there is a return of the chromatin, and of the asters to the interior of the egg.

The stages that furnish the most instructive results are those during the period of formation of the segmentation spindle and just before the cytoplasmic division when the chromosomes have separated and begun to form separate vesicles. When eggs with a segmentation spindle are strongly centrifuged they may be driven out into bottle-shaped forms or into strings. The spindle lies just above the yolk-ball, where the polar half normally constricts from the antipolar hemisphere. Consequently this spindle becomes caught in the elongation and may suffer distortion. Such a case is shown in figs. 41-43, where the fibres of the poles are thrown into curves or spirals, and the central spindle bent in its middle between the plates of chromosomes, fig. 42. This figure shows that the rays are not broken but bent and their semisolid nature made evident. The "neck" is narrower than the actual size of the segmentation spindle, hence in order to be contained in this space the long fibres are bent. It should be recalled that at this stage the astral system has reached its maximum, and the rays fill almost if not quite the entire egg.

When the chromosomes reach the poles they become individually vesicular and later these fuse into a single nucleus near each astrosphere. At this time just prior to the cytoplasmic division the effect of the centrifuge is to carry the vesicles towards the pole, and since these are closely connected with the astral system that system is dragged towards the pole. The results produce effects like those shown in plate 8, figs. 51, 53. These stages show the group of vesicles or the two nuclei, if the vesicles have fused, beneath the polar cap. From them streamers representing the

astral rays extend into the egg. In fig. 48 the two new centers for the second division have been formed and lie in a bay of the nucleus. They have been carried with the nucleus to the top of the egg. In this figure some of the fibres appear to be bent in such a way as to change their course by 90° but owing to the confusion of fibres in this region there is a chance of a misinterpretation of the real state of affairs.

The nature of the cytasters and spindle

Concerning the nature of the forces at work that produce the aster these experiments give no definite information, but they throw a good deal of light on the nature of the aster when it is formed. The fibres of the new aster appear more rigid than those of the older aster for the former appear seldom bent when the protoplasm about them is moved. The bombardment of the aster by the yolk granules tells the same story; for, the yolk granules filter through the aster without affecting it. Those only are caught that are carried down the funnel-like lines of the converging fibres. The older fibres appear more often bent either because they are more flexible, or because they extend further into the cytoplasm and are more affected. Perhaps both conditions are involved. On coming in contact with the wall of the cell the fibres may be bent, and a similar phenomenon is seen when the polar spindle passes to the surface of the egg. In the latter case, however, the bending might be supposed to be due to an active movement of the fibres, while such an interpretation seems excluded in the centrifuged eggs.

The results show that the asters may be carried bodily through the cytoplasm without distortion. This fact creates at least a presumption in favor of the view that in the normal egg the movement of the aster or karyokinetic figure is due to a passive transportation through the cytoplasm, and that the movement is not due to a continuous dissolution or reformation of the aster in the direction of its movement. This conclusion has a bearing also on the nature and formation of the astral system.

If the asters are supposed to be the outcome of forces emanating from the centrosome or centrosphaere and if the maintenance of these fibres is due to the same activity, it is difficult to understand how the fibres could become bent and maintain themselves for a time in this form. The preparations give every indication that the fibres are real although temporary structures. The observations give little evidence that could be used to interpret the rays as lines of force.

The rôle of the astral system in cell-division

If the astral rays are relatively rigid and if the system is moved passively through the egg, and does not move by its own activity, the process of cell-division can not well be attributed to the activity in a dynamic sense of the fibre system. The change in position of the asters must, therefore, be the result of general movements in the cytoplasm that carry the asters with them.

It is notorious nevertheless that the asters, or the poles of the spindle, when they come sufficiently near to the surface of the egg act in some way as centers around which the division takes place. It has been shown by Teichmann and by Wilson that asters representing the poles of neighboring spindles may also incite a plane of cytoplasmic division to pass through them. Evidently it is not necessary that a spindle containing dividing chromosomes shall be in the middle between two centers. This fact in itself makes improbable the view that the chromatin or chromosomes take any active part in division,² and consequently those theories, such as the recent one of Loeb, that rest on the assumption of some special activity emanating from the nucleus are probably incorrect. The same conclusion is made probable from those cases where the division plane falls entirely to one side of the chromatin so that one cell gets all the chromatin and one aster; the other cell gets only the other aster. Such a process cannot be interpreted on the basis of the nuclei as the focal points necessary for division.

² Except in so far as the development of the central spindle brings about a sufficient separation of the poles to induce segmentation between them.

Still another observation points in the same direction. In some eggs—the frog's for instance—the first division may slowly pass through the yolk hemisphere, while the preliminary steps for the next division are going on in the region of the nucleus. I have pointed out that the spindle for the second division may actually develop before the first division is completed. Evidently protoplasmic division once begun may continue when the nucleus has passed or is passing into its next phase. Such facts are difficult to interpret on the assumption that the nucleus is the active center of the change. But in these eggs the peripheral astral system may remain and possibly continue its development into remote parts after its original center has divided into two. The presence of this peripheral system may suffice to complete development once begun. Yatsu's experiments with egg fragments point to the same conclusion. Other evidence might also be adduced to emphasize the importance of the astral system in division but this will suffice to show its significance. How then shall we interpret the action of the aster? Two suggestions offer themselves for consideration: First, the system of rays stiffer than the rest of the cytoplasm may represent a skeleton or supporting frame work for the mechanism involved in the division. Such an interpretation would still leave the essential features of the process to be explained. Second, the formation of the rays may cause a change in the surface tension of the cell leading to a constriction of the mass about the astral centers. If, as I have suggested, the formation of the rays represents a molecular change in the ground substance, such a change might involve an alteration of the interior of the cell in contrast with its surface, and from a physical point of view such changes taking place around two centers might be made to account for the constriction of the cell. Whether these ideas have any value as working hypotheses the future must show. The observations here recorded suggest at least the possibility of analysis along these lines.

The specific gravity of the spermatozoön

The sperm head is sometimes spoken of as the condensed nucleus of the spermatid. If by condensation is meant a more solid, *i.e.*, less watery consistence the centrifuge would give evidence of the fact, for by its means bodies having differences in weight are sorted out. The evidence shows that the sperm head remains in that part of the egg where it enters and therefore is not either heavier or lighter than the general cytoplasm. The failure to move the chromosomes of the egg points in the same direction, but their attachment to the spindle might prevent their movement unless their weight were sufficient to drag the asters with them. Yet even at the dissolution of the nucleus, and before the fibre system is fully developed, the chromosomes can not be moved in any of the eggs that I have studied. As soon as the sperm head begins to become vacuolated its transportation in the cytoplasm is made possible. This is due beyond doubt to the material contained in the vacuoles being lighter than the cytoplasm. The watery nature of some of this material is therefore probable.

The head of the sperm contains in addition to the original cell-nucleus the condensed cytoplasm of the spermatid. An examination of the formation of the spermatozoön shows that the cytoplasm shrinks around the elongated nucleus, but there is no evidence that any of the cytoplasm is lost. Its diminution would seem to be due to loss of watery fluid, and if the presence of a more watery fluid in the nuclei makes them lighter its loss in the cytoplasm would increase the weight of the latter. If then the surrounding pellicle of the protoplasm of the sperm is denser than ordinary cytoplasm this element should increase the chance of the sperm centrifuging to the heavier pole. But, as stated, there is no evidence of such transportation.

On the other hand the comparison includes cells from different organs of different individuals, and even if the sperm is denser than the spermatid it need not follow that it is therefore denser than the egg. The evidence shows however that if there is any difference it is not shown by a centrifugal force sufficient to separate other constituents of the egg no larger than the sperm head.

Comparisons with the Chaetopterus egg

Lillie's description of the egg of the annelid, *Chaetopterus*, and of the effects of centrifuging it shows a remarkably close similarity between these eggs and those of the nemertean *Cerebratulus*. Lillie's explanation of the formation of the yolk-ring in *Chaetopterus* applies directly to the similar ring in *Cerebratulus*, and the presence of small basic microsomes in *Chaetopterus* that collect at first at the top of the yolk-ring also finds a counterpart in *Cerebratulus*. The comparison extends even to the presence of a superficial ring of yolk-granules imbedded in a denser ectosarc. The similarities between the cleavage of nemerteans and annelids have been pointed out by Wilson.

In this connection it is not without interest to find that in both forms the polar spindle can be moved from the pole to the center of the egg, should the pole be turned outwards on the centrifuge. The regularity of the maturation process in *Chaetopterus* offers a better opportunity to study the problem. I have some preparations of the eggs of this species that confirm in every respect Lillie's interpretation. It is a matter of so much importance for subsequent interpretation to make certain that the eggs do not orient that I shall consider the evidence in some detail.

The polar spindles are, after centrifuging, found in the cytoplasm and only rarely in the yolk hemisphere. If we assume that the eggs orient on the machine so that the pole near which the polar spindle lies moves centrally, we should expect the results to be as we find them, *i.e.*, the polar spindle in the cytoplasmic half. But if the spindle has fully formed before the centrifuging begins, and has reached the surface, we should still expect to find it near the surface and oriented radially, except in so far as it is forced in by the presence of the oil cap. In fact, only about half of the spindles of the centrifuged eggs lie near the surface and radially, the rest lie anywhere in the cytoplasmic hemisphere and often near or even in the yolk. This condition would result if in all those cases where the spindle lay in the outward hemisphere it was displaced by the yolk. This, in fact, is Lillie's interpretation. It seems to me valid. The only argument opposed to

it is that the development of the oil-pole may be responsible for the movement of some of the spindles into the interior. In fact Lillie thinks that the oil-pole may cause such a shift, but he implies that in such cases the spindle still keeps its radial position. There is another way, free from this objection that has not, it appears, been used to settle this question. If centrifuging is delayed until the first polar body is formed, that body should be found as often in the yolk hemisphere as in the light hemisphere, unless, in fact, the presence of this body acts as a load to turn the egg, as seems to occur to some extent in *Cumingia*, but the egg of *Cerebratulus* is so large that it may not be affected by the polar body. Different eggs should give different results in this regard.

If the eggs are revolved either before the polar spindle forms or when it is still present, those polar spindles that come to lie in the interior must penetrate the yolk sphere once more in order to reach the surface. Should the yolk-sphere be too dense to permit this we have the anomalous condition found in both *Cerebratulus* and *Chaetopterus*.

What will happen to the delocalized spindle? If it fails to reach the surface again half the eggs should fail to extrude their polar bodies. Lillie does not state specifically that he has observed this in *Chaetopterus*, but his statement, that centrally lying spindles are found later, implies that polar bodies had not at that time been given off. The fate of these spindles and the history of such eggs is not described. I have already considered these questions for *Cerebratulus* and need not repeat here what I have there said.

Lillie's conclusion in regard to the movement of the spindle in *Chaetopterus* has certain consequences that he has overlooked or else refrained from discussing. If, as he has tried to show, the centrosome is a "center of force" that produces the astral rays then when the centrifuge moves the karyokinetic figure as a whole through the egg the rays must form and reform at each step in its progress. When one considers all the consequences of such an assumption he will admit, I think, that such an interpretation is highly improbable.

The evidence on which Lillie bases his argument that the centrosomes are centers of force also rests, I believe, on a very un-

stable basis. The point has been fully discussed in my account of *Cerebratulus*. Here I need only add that while I agree with Lillie that the centrifuge offers an opportunity to settle which alternative hypothesis—center of force versus the mitome theory—is correct,³ my own evidence points to the latter as the more probable interpretation.

There is, in fact, no need to have recourse to the centrifuge to show the relation of the spindle fibres to granules. When the germinal vesicle breaks down, the fibres that appear in its area show granules arranged in irregularly bead-like rows.⁴ Through the courtesy of Professor Wilson I have examined preparations of the egg of *Thalassema*, where the formation of the fibres is clearly shown. The fibres that extend out into the cytoplasm are largely free from granules, while those that develop within the area of the germinal vesical are covered with granules especially at their outer ends. Their later disappearance suggests the possibility that they may become incorporated within the fibre and even form part of its clear material. Such a possibility does not however involve the conception of the center of force hypothesis.

Comparison with the frog's egg

It is not my wish to enter into a full comparison between the results here recorded and those which others⁵ and I myself have obtained with the frog. Only two or three points will be considered.

The egg of the frog when set free from its inner membrane rotates and orients on the machine. But if fixed, the contents may rotate as a whole beneath the denser outer ectoplasm. Gravity suffices in time to produce this result on eggs that have

³ These contrasts are those that Lillie has drawn. By rejecting the first I do not mean to commit myself to the second further than my discussion of the aster-formation implies.

⁴ See the excellent figures of Griffin, B. B., Jour. Morph., 15, 1899.

⁵ I am taking for granted in this discussion that the fibres of the aster are not artefacts, but I am far from wishing to deny that in the living egg these fibres may be little more than denser tracts of hyaloplasm.

⁶ The recent papers of Gurwitsch and Kqonopacka and McClendon relate to topics other than these here discussed.

absorbed water—*i.e.*, not on ovarian eggs,—and consequently centrifuging, especially if slow, may do the same thing. There is a consequence of some importance involved in this conclusion. If the contents revolve as a whole it retains (*i.e.*, it carries with it) the former polar or molecular structures. Consequently the polar bodies may be given off even after rotation through 180° and the cleavage may begin at the light pole, and produce there small cells. The failure of the ectosarcial layer to take part in the movements of the interior may lead one to a false conclusion, if a comparison between the frog and other forms is made, unless this factor is taken into account. In other words it may appear that the polar bodies of the frog's egg may be given off at any point of the surface, if the immobile ectosarcial layer be taken as evidence of polar relation, while in reality the rotation, *en masse*, of the contents beneath the surface may be responsible for the results. Other eggs than the frog's may be expected to give essentially the same results.

3. CENTRIFUGING THE EGG OF HYDATINA

Whitney has shown for the eggs of *Hydatina senta* that the typical three zones appear after centrifuging, and that normal embryos result irrespective of what part of the egg contains these materials. Since the egg is not fertilized, and since the polar bodies are given off at the side, this egg offers exceptional opportunities for the study of the relation between the visible substances in the egg and development. The results corroborate in every way those obtained for the sea urchin, *Cumingia*, frog and other forms.

The question has been raised whether the effects of centrifuging may not be different at different periods in the maturation of the egg—*i.e.*, differences may result according to whether the separation takes place before or after the breaking down of the germinal vesicle. That differences may exist is abundantly shown from the evidence here adduced concerning what the centrifuge may do in regard to the nucleus and spindle, to take but two examples. These questions, however, do not touch the real point

at issue concerning the so-called formative stuffs. There is one matter, however, of special importance. It may be claimed, and has, in fact, been intimated, that a redistribution of the centrifuged materials may take place so that the first separation is not present later. In fact mixing does occur, due in part to the entrance of the sperm, but primarily to the movements instituted by the karyokinetic division. But if the eggs are centrifuged during the division this remixing can be counteracted, and in any case its importance has been exaggerated. At least, I find little or no evidence that the different materials return to their former positions in the short time elapsing before cleavage. In unfertilized eggs the distinctions are as great, if not quite so sharp after 24 or 48 hours as they are at first.

In order to meet, as far as possible, criticism of this kind, I have made some experiments with the eggs of *Hydatina*. Immense numbers of individuals were collected at the surface of the culture by the method that Whitney worked out. These were put into shallow watch crystals, and poured off after five minutes or less. A few eggs are generally laid during this time. These were centrifuged at different intervals after laying. The time of the first cleavage, and its location in relation to the "stuffs," was noted; and this record gave a further control on the conditions of the egg when centrifuged.

The results may be stated in a few words. *Normal embryos were obtained irrespective of the time when the centrifuging took place.* In some instances division followed almost at once on removal from the machine and normal embryos resulted. There could have been no question of a redistribution of materials in such cases. At first the eggs were put into spring water. Here they developed and often hatched, but the individuals, although, sometimes normal in structure, appeared still and dead. Such evidence might readily have led to the conclusion that the centrifuging was the cause of this premature death. Such a conclusion would have been entirely wrong and shows how careful one should be in drawing conclusions concerning the injurious effects of the separation of the materials by the centrifuge. Cumingia taught the same lesson from another standpoint. I found

that if the eggs of *Hydatina* were put into the filtrate of the original solution that they hatched into normal individuals, which in time laid normal eggs. Evidently the sudden change from the old solution to the new one, while not affecting the egg within the membranes killed the individuals after they had hatched.

The details of some of the cases are given in the following table.

TABLE 1

NO. EGGS	LAID	CENTRI- FUGED	DIVIDED	RESULTS
1	11-11-20	11-35	12-20	Normal but body bent to one side.
20	3.03- 3 08	3-30	All but one normal.
5	11-25-11-35	12-10	12-10-15	Normal. Four hatched slightly abnormal especially at one side.
3	11-25-35	12-10	12-12	Hatched but not normally active.
14	3-20-30	4.00	4-15	None hatched.
8	3-20-30	4	4-15	Three hatched.
9	11-30-40	11-20	In 2-cell when taken off.	Five hatched; normal.
7	3-10	3-40	3-50	Four hatched; normal.
2	11-10-20	11-10	1 going 2-cell; other not yet.	Both hatched; normal.
	3-10	3-40	4.06	Normal.

Some of the above series were carried out in spring water; hence the mortality and abnormality especially in the first and fifth record. Failure to hatch can be also ascribed to the same factor in several records. The positive cases suffice to show that normal embryos may appear without regard to the time of centrifuging, and individual records confirm Whitney's observation that these normal embryos belong to all the types of distribution of the visible materials of the egg.

4. CENTRIFUGING THE EGG OF THE FISH

The distribution of the yolk and protoplasm in the egg of the fish is so different from that in other eggs that have been centrifuged, that its study presents some points of peculiar interest. The protoplasm lies at first in a clear zone over the periphery of

the yolk sphere. When the mature egg is set free in the water the protoplasm moves to one pole to form there the blastodisc. The blastodisc lies immediately below the micropyle, which is the only point in the membrane through which the spermatozoön can enter. The protoplasm is transparent, and almost entirely free from granules, which are so characteristic a feature of the eggs of other animals.

A cursory study of the effect of centrifuging sufficed to show that the blastodisc can be produced at once when the eggs are centrifuged, instead of taking an hour to form as in the normal egg. The blastodisc appears on that part of the egg that is turned outwards on the machine. It is clear and transparent as in the normal egg, and shows no stratification of its substance. Its location bears no relation to the micropyle, which raises at once the question whether the blastodisc is produced at any point on the surface of the egg that happens to lie outermost, or whether the egg proper orients within its membranes in response to the centrifugal force. Simple as these alternatives appear, no small amount of labor has been necessary to settle this point. The solution of this question seemed the more desirable because it not only involves certain important problems in the egg of the fish, but bears on certain possibilities in the eggs of other animals.

Another point of interest appeared when it was found, that, beneath the micropyle, a minute blastodisc also appeared after fertilization, that segmented and produced a rounded blastoderm. The relation of this blastoderm to the larger one, its origin, and its fate have also been examined.

The work had progressed this far in the summer of 1908, but wishing to settle more conclusively certain essential questions, I repeated and extended the experiments during part of the summer of 1909, and am now in a position to give an answer to the main problems outlined above.

The orientation of the egg in the machine

An examination of unfertilized eggs placed at once on the machine and turned 150 times shows that the blastodisc artifi-

ally produced may lie in any relation to the micropyle. It was important therefore to determine whether the artificially produced blastodisc is formed in a different region of the egg from that occupied by the normal blastodisc, or whether the result is due to the turning of the egg within its membranes.

It seemed to me that if serial sections were studied there would be no difficulty in determining whether the egg rotated within its membrane, or whether the blastoderm formed at any part of the surface. The nucleus of the normal egg lies in that part of the protoplasmic envelope immediately beneath the micropyle. Agassiz and Whitman have shown for this same egg that the protoplasmic shell is thickest at the micropyle. They record for the egg of *Ctenolabrus* that at this region the protoplasm is thickest thinning out gradually towards the opposite pole where it is very thin. If the artificially produced blastodisc corresponds in position with the natural blastodisc, we should find the egg nucleus, or the polar spindle, at or near the center of the artificial blastodisc. But if the blastodisc forms in a new place it might be without a nucleus at first, or the nucleus might often lie in an eccentric position in the new blastoderm. If the polar spindle had developed before centrifuging, it might fail to be carried into the blastodisc, or if transported, it might lie at first obliquely at the periphery of the blastodisc.

Serial sections were prepared of a number of eggs in different stages. The preparations were excellent and the series in most cases complete; yet I have searched in vain through some of the series to find either nucleus or spindle. I have found the egg nuclei or conjugating nuclei in only seven eggs; in two cases the polar spindle was formed. In all of these cases the nucleus or spindle lay exactly in the middle of the blastodisc. The failure to find the nucleus or spindle in other cases I attribute to the following conditions. The polar spindle of the fish egg is extremely small, and easily overlooked; any disturbance in the protoplasm such as that produced by the sudden heaping up of cytoplasm might make the discovery of the spindle still more difficult. Whether, in fact, the spindle would be buried in the mass of cytoplasm or lifted to its surface, I do not know, since in one case the

spindle was imbedded, and in the other was on the surface; but in the latter the centrifuging was not hard and the blastodisc still flat. The resting nucleus is not sharply marked off from the cytoplasm, and is difficult to detect, owing to the presence of vacuoles in some of these centrifuged eggs. In many cases the egg is filled with them; in other cases they are scarcer—owing to differences perhaps in the state of maturity of the egg. As the centrifuging was done soon after the egg was stripped from the fish it is possible that the germinal vesicle may have just broken down, which would render the detection of the chromatin much more difficult. It would have been of no avail to wait before centrifuging until the easily detected segmentation spindle formed, because at this time as is well known the egg may move readily within its membranes. Finally, although I have looked for the nucleus or spindle in other parts of the surface of the egg I have never seen them there. It would be most laborious to thoroughly search the periphery of every section, and since the eggs were punctured in order to imbed, the absence of discovery might be attributed to the nucleus lying near the puncture.

After centrifuging, the superficial cytoplasm, except at the blastodisc, is reduced to a minimum. If a spindle formed elsewhere than in the blastodisc, an accumulation of protoplasm would be expected to occur, but I have seen none. I am inclined therefore to think that the failure to find a nucleus in the blastodisc in many eggs is due to technical difficulties, not to its absence. Nevertheless, despite the time spent in this side of the problem, the outcome is on the whole negative, since there are too many failures to find a nucleus for the positive cases to count for much, as such cases might be attributed to a coincidence. I therefore returned to the experimental side once more, where, fortunately, I have obtained convincing evidence that the egg may move as a whole within its membranes. The essential part of this evidence will be given in the three following paragraphs.

1. If the blastodiscs be artificially produced, and the eggs turned over and over so that they are no longer orientated and then again centrifuged for a few turns, the blastodiscs orient at once. The experiments show clearly that the eggs orient within

their membranes readily after the blastodisc has begun to form. It still remains to be shown whether the same orientation occurs before the blastodisc has begun to thicken. Owing to the difference in thickness of the outer protoplasmic layer, it is possible to settle this question also by direct observation. If unfertilized eggs are put into a tube and rotated so slowly that no change in the protoplasmic layer is effected, and the tube containing the eggs be examined under the microscope (care being taken to keep it in the same position it held on the centrifuge) it will be found that the eggs orient within their membranes.

2. If the eggs soon after removal are shaken about in seawater it will be found that in many cases the thickest part of the protoplasm no longer coincides with the micropyle. This shows how easily the egg shifts within its membranes.

3. If, after centrifuging, the egg nucleus or the spindle were left behind in their normal positions when the rest of the protoplasm was carried into the artificial blastodisc, we should expect to find after a time a certain accumulation of protoplasm around the nucleus, to judge from analogy with what occurs around the sperm nuclei, but none becomes apparent. For instance, eggs were centrifuged to various extents, and then left to stand for an hour or more without fertilization. No evidence of any accumulation of protoplasm, except in the region of the blastodisc, was found.

From the foregoing evidence there can be no doubt, I think, that the eggs orient within their membranes under the influence of a strong centrifugal force, and that the egg membranes do not always turn when the egg turns, owing in all probability to the pressure of the eggs on each other. If floating freely in the water, however, the membrane and the egg usually turn at first together as I have seen.

*The effect of centrifuging the eggs immediately after removal
from the fish*

When just out of the fish the surface of the egg is in intimate contact with the inner surface of the membrane, so that the egg

turns as a whole unless roughly handled. In consequence the spermatozoön entering the micropyle passes by the nearest path to the center of the future blastodisc.

Contact with sea-water causes the surface of the egg soon to separate somewhat from the inner surface of the membrane, so that the egg may turn within the membrane. Any sudden or irregular movements of the egg at this time may cause the egg to shift, so that the center of the blastodisc no longer corresponds with the micropyle. Several years ago I showed that if the egg of the fish is not fertilized within a few minutes after removal to sea-water, polyspermy occurs, and in the light of the facts to be recorded this result is, beyond doubt, connected with the rotation of the egg within its membranes. The process of "dry fertilization" so often recommended by pisciculturalists owes, perhaps, its success to the fact that in the absence of water the separation of egg and membrane may not occur until after fertilization; hence a larger percentage of normal embryos.

Despite the close contact between the egg and its membrane, the centrifuge causes the egg to rotate as a whole within its envelope, as stated above. In consequence another part of the egg surface comes to lie beneath the micropyle. In order to reach the artificial blastodisc the sperm, entering through the micropyle, must pass into a foreign part of the protoplasm, and then to reach the blastodisc must be transported over the surface of the egg in the thin layer of protoplasm. The consequences of this disjunction of normal conditions lead to several results.

In the first place the results show that spermatozoa may enter at any part of the surface, and that even if there were a localized region of the protoplasm for the entrance any other region does as well, so far as mere entrance alone is concerned; but in regard to polyspermy the results are different. There is evidence that several spermatozoa enter in most of the centrifuged eggs. The first that enter move or are carried by the shortest path towards the blastodisc. Those that enter later remain near the micropyle, where a small accumulation of protoplasm appears. The most probable interpretation of these facts seems to be that the occluding reaction in the egg, by means of which accessory or

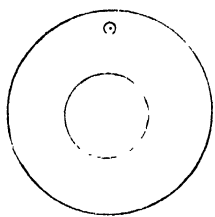
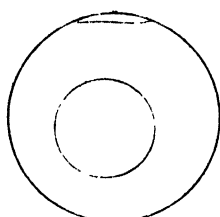
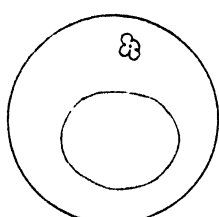
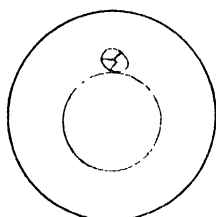
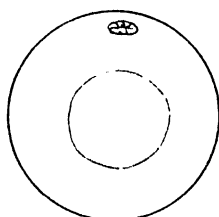
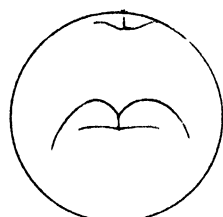
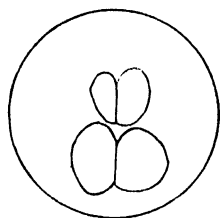
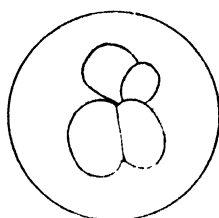
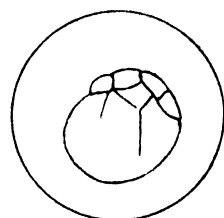
supernumerary sperm are prevented from entering, does not take place so quickly when sperm enter at other regions than the normal one. If we think of the spermatozoa as boring into the surface of the egg, we must suppose in the present case that they continue to do so until the occluding reaction has occurred. If on the other hand we think of the entrance of the spermatozoön as involving a responsive reaction on the part of the cytoplasm, we must suppose in the present case that this reaction persists until the first spermatozoön having reached the region of the blastodisc, the occluding reaction sets in and prevents further entrance. Possibly the result may be more simply explained on the ground that the peripheral protoplasm is so thin that the reaction is insufficient to occlude for some time the entering sperm.

If the egg is first fertilized and then centrifuged, the microblastodiscs do not appear. This shows that the occluding reaction is effective, when normally carried out, not only for the region of normal entrance, but for all the rest of the protoplasm as well.

The transportation of the sperm at times over an extensive region of the egg in a thin layer of protoplasm is one of the most remarkable phenomena that I have to record. Until we know more about the cause of the movement of the sperm nucleus it is idle to speculate on this case; but the fact of its transportation over a long distance in an attenuated layer of protoplasm is worthy of remark.

The cleavage of these eggs offers many anomalies. In a few cases the blastodisc divides normally into two equal cells. These, and only these eggs, produce normal embryos, as I have determined by isolating them. Most of the eggs break up irregularly into several cells (fig. G, H, I) resembling in every respect the abnormal segmentation observed in eggs with delayed fertilization, and, like the latter, produce abnormal blastoderms.

Some of the eggs show a more peculiar relation. The main blastoderm divides regularly into two cells, while at one edge a small independent microblastodisc also divides. These two may later intimately fuse. From such eggs some of the most interesting types of embryos that I have seen may be supposed to have come. These embryos are defective on one side (figs.

*A**B**C**D**E**F**G**H**I*

O, P, Q, U) to various degrees, the defective side being that derived from the region of the microblastodisc. The details of structure of these embryos will be described later. The behavior of the blastodisc during cleavage I interpret to mean that the first sperm nucleus entering from the blastoderm brings about normal fertilization. Others entering later are carried to the edge of the blastodisc, and failing to enter, produce a small blastodisc at one side. The presence of this blastodisc interferes with the normal development of the blastoderm. In other cases many sperm entering at about the same time are carried into the blastoderm, and produce there the polyaster that gives the multiple cleavage. No embryo, or even a part of an embryo, forms under the latter conditions.

I was not without hope that the microblastodisc might give rise to micro-embryos, paternal in origin, but although I have had hundreds of such blastodiscs none have produced embryos. Most of them owe their origin to more than a single sperm, and hence are probably derived from polyastral figures. Under such circumstances normal development might not be expected judging from the behavior of the large polyspermic blastoderm. But in some cases, where the microblastodisc divided at once into two, it is probable that a single sperm is responsible for its formation and cleavage, yet I have obtained no embryos from these microblastodiscs, when eggs containing them were isolated. I can only attribute this failure to the minute size of the mass; for, to judge from what happens in uninucleated pieces of the sea-urchin eggs, paternal embryos are possible formations.

Thinking that the smallness of the blastodisc might be due to the paucity of protoplasm in its vicinity I tried the effect of centrifuging no more than was enough to move the egg within its membrane, and not enough to carry the peripheral protoplasm into a disc. The results were the same as before, and the slight difference in size of the microblastoderm, if such differences exist, produced no effect, for the greater part of the protoplasm flowed into the large blastodisc.

I was also not without hope that the sperm entering at a remote point from the artificial blastodisc might fail to reach the blasto-

disc, and yet excite in it those changes that would lead to its development. In such a process the embryo would be purely maternal—owing only to the male its impulse to begin its development, but none of its material. The evidence of many sections goes to show, however, that the spermatozoa are carried into the blastodisc, and that one at least unites with the egg nucleus as in normal fertilization.

The method by which the protoplasm is drawn into the blastodisc

It has long been known to students of fish development that after deposition there is a steady movement of the peripheral protoplasm of the egg into the blastodisc. The cause of this movement is entirely unknown and seldom discussed. It is not, however, a unique phenomenon, for something like it seems to occur in other eggs, although not always in the same direction, or under the same conditions. Conklin has given some very full and accurate descriptions of such movements in the eggs of several gasteropods. In some of these the movement seems to be connected with the dissolution of the germinal vesicle in consequence of which the protoplasm becomes more fluid. In the ascidians the movements are connected with the entrance of the spermatozoön. In the fish the latter event cannot be the cause of the movement, since it takes place in the unfertilized egg; and it is improbable that the dissolution of the nucleus is its chief cause since the movement goes on throughout the entire time of polar body formation and conjugation of the pronuclei, and even during the early cleavages. The concentration is not connected with the absorption of water by the egg to make the protoplasm more liquid and no swelling of the membrane to set free the interior takes place as measurements, that I made, show. The surface of the egg is under high pressure as seen by sticking the egg, when the yolk rushes out through the opening. The enveloping protoplasm shrinks, but the membrane remains as before. By flattening the egg between glass plates the yolk often breaks inside the membrane through the surface layer of protoplasm of the egg, and escapes into the space between the membrane

and the shrunken egg. At the same time the protoplasm with the remaining yolk shrinks into a small sphere. If we could account for a decrease of pressure over one pole in the region of the blastodisc, the movement observed may be accounted for, since the protoplasm, like any other fluid, will flow from a region of higher pressure to one of lower. The micropyle is the only direct communication with the outside. Through it water might escape, but that the formation of the normal blastodisc is not due to the presence of the micropyle above it is shown by the formation of the blastodisc elsewhere when the eggs are shaken so that the egg is turned inside its membranes.

The centrifuge increases the pressure on that pole that is turned outwards in the sense that the protoplasm being heavier than the yolk in the fish's egg is thrown more towards the outer side of the egg. In consequence the centrifugal force accomplishes in a minute that which it takes the normal egg an hour to bring about. This fact may throw some light on the nature of the normal phenomenon that has been so difficult to explain. The distribution of the protoplasm on the surface is due to the stretching of the peripheral protoplasm by the yolk-sphere pressing it against the membrane. If the pressure were released the protoplasm would flow towards that region where it is already thickest. But what releases the pressure? If substances are set free from the protoplasm so that it shrinks, the formation of the blastoderm could be explained. Agassiz and Whitman describe the disappearance of watery spaces in the protoplasm. These may, it is suggested, produce the fluid that comes to lie between the egg and its membrane.

If our analysis is correct for the normal process we see that the centrifuge brings about its results by the same method as that of the normal egg, and the physical phenomenon is the same. The centrifugal force by driving out the fluid from the vacuoles in the protoplasm hastens the formation of the blastodisc.

Is gravity a factor in the formation of the blastodisc?

The blastodisc appears on that part of the normal egg that is turned down or outwards on the centrifuge. The normal orientation of the egg, as it floats in the sea, is due to gravity. Does gravity acting in the same way as does the centrifuge besides orienting the egg also produce the blastodisc?

I tested this possibility by placing the eggs in a long test tube half-filled with water. A bicycle was turned upside down and a jet of water from a faucet allowed to run over the outer edge of the tire of one wheel, which caused the wheel to turn 10 revolutions per minute. At each revolution the water and eggs fell twice from one end of the tube to the other so that the eggs were kept continually whirling irregularly in the tubes and were practically never at rest. The eggs were fertilized at once and placed on the wheel. The eggs were kept on the wheel until the two and four cell stage appeared. They were then removed and developed normally.

The blastodisc of the rotated eggs resembled the normal one in every respect. Evidently gravity is not necessary for the formation of the blastodisc even although the direction of its action is the same as the direction of the centrifugal force of the machine.

In the light of the discussion between Moszkowski on the one side, and of Katheriner and myself on the other, concerning the rôle of gravity in the development of the frog's egg, it is not without interest to note that these rotated fish eggs developed in a perfectly normal way. Moszkowski claimed that the action of gravity is essential for the normal development of the frog's egg. This was denied by Katheriner and myself on the basis of experiments carried out to test the question. The results on the fish show that in this egg also the action of gravity in a constant direction is of no importance for normal development.

Abnormal embryos after centrifuging

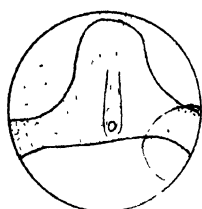
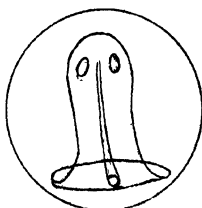
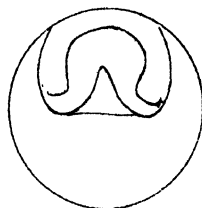
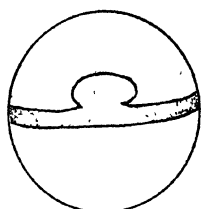
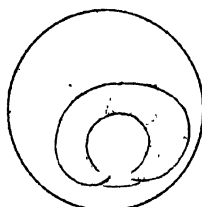
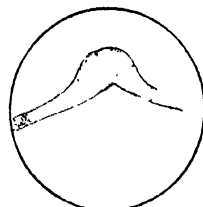
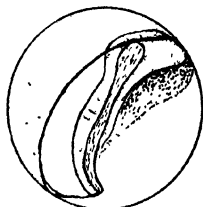
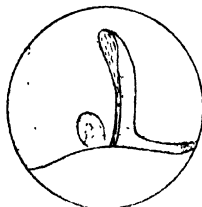
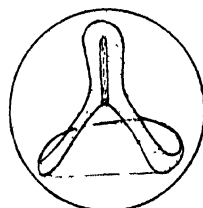
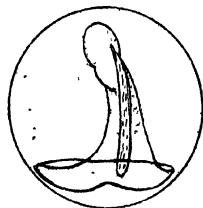
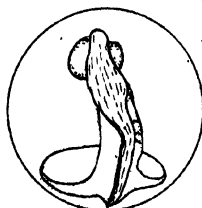
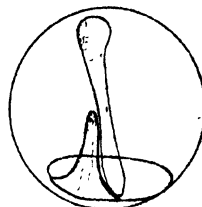
Since the centrifuge causes no stratification of the materials of the egg of the fish (except in so far as it facilitates the formation of

the blastodisc at one pole) and since the egg orients on the machine, the abnormalities that so frequently appear must be ascribed to some other cause than separation of materials. This other cause I have discovered—polyspermy. If eggs are first fertilized, and then revolved they produce normal embryos even, when the eggs are left in the machine until the cleavage furrows have appeared. But if the eggs are first centrifuged, and then fertilized, polyspermy follows in a majority of the eggs. Only those produce embryos, as explained, in which one sperm nucleus combines with the egg to give a normal bipolar karyokinetic figure.

The following diagrams show some of the more common types of embryos. They are not peculiar to centrifuged eggs, but may be found in polyspermic eggs that have arisen from any other source.

In fig. J a young nearly normal embryo is shown, but at one side a separate blastodisc is present, which has not, however, interfered with the formation of the embryo up to this stage. In fig. K the embryo, although elongated, has failed to draw together in the middle line. In fig. L the anterior end only of the embryo has developed. The medullary plate is split open widely behind—only a thin layer of cells covers the V-shaped split. In fig. M the embryo has hardly differentiated at all, but appears as an enlargement at one point on the germ-ring. Such an embryo furnishes a step towards the condition when the germ-ring alone is present.⁷ In fig. N the embryonic material has the form of a horse-shoe owing most likely to the paternal portion of the blastoderm lying at the posterior end of the embryonic region. In consequence the blastoderm fails to concentrate at this side to produce the primordium of the embryo. In fig. O the beginning of the embryo is shown, but one side is very incomplete. If an embryo were to develop later from such a beginning it would have only the head and one side of the body present and would probably resemble the embryo shown in the next figure. This embryo, fig. P, is strictly a half-embryo except in the head region that is more nearly whole. On the defective side there is a mass of unor-

⁷ See Morgan, *The Formation of the Fish Embryo*, Jour. Morph. 10, 1895.

*J**K**L**M**N**O**P**Q**R'**R**S**T**U*

ganized cells, and these have no doubt been the cause of the lack of development on this side. The next embryo (fig. Q) is also defective on one side, at least as far as concerns the mesoderm. A small lump at the edge of the overgrown part represents, no doubt, the defective material of the missing half. In fig. R the posterior end of the embryo has failed to advance over the yolk at the same rate as the germ ring. In consequence the embryo has remained short and thick. A later stage of this embryo, when the germ ring has nearly closed, is shown in fig. R^I. The elongated blastopore occupies the region normally taken up by the elongated embryo. It is not without interest to find an elongated slit, showing as it does that the anterior lip of the blastopore, after reaching a certain point, fails to advance further. In fig. S a defective embryo is shown in which the head is twisted and the posterior end spread out widely. Similarly the embryo in fig. T is defective in several respects. Finally fig. U shows an embryo in which apparently the nerve cord and notochord have developed side by side instead of above and below.

These embryos, after drawing, were set aside to be studied by means of serial sections, but much to my regret the bottles containing them were lost in transportation. The main features at least are apparent from the surface views.

These embryos have, I think, some general interest in connection with the question of the mode of formation of the embryo, whether by elongation or concentration, or by concrescence. I have looked in vain among all the embryos with wide open blastopore for evidence of differentiation of the material of the germ-ring, which would be expected on the concrescence theory. I have never found any evidence of such development. The nearest approach to such a condition is shown in the half embryo of fig. P, but here the mode of formation is clearly of another sort. Owing to injury of one side of the blastoderm the embryonic knob has extended backward along the middle and one side, while the material of the other side has been largely held back in consequence of the defective condition of that side. These embryos support the conclusion that I reached by experimental methods in 1895 which Kopsch confirmed later; namely that in the teleost the germ-ring does not

represent the sides of the embryo as His supposed, but that the material for almost the entire embryo is laid down in the early embryonic shield, and draws together and is carried backward as the germ-ring advances. A small contribution from the germ-ring probably occurs in the later stages of closing. This interpretation is entirely in accord with the evidence furnished by these embryos.

In several respects these embryos are comparable with those that I have described for the frog's egg after centrifuging. The defects are, however, due to different factors —here polyspermy, there concentration of the yolk.⁸ The cause of the defect is of small importance compared with the interference that defective regions exert on the course of development.

⁸ In my paper on The Centrifuged Frog's Egg, I referred these defects to injuries to the yolk field. Here I adopt McClendon's amendment, namely, that the yolk hemisphere is not injured, but made unmanageable. To this amendment I will add that since in my experiment, the eggs were kept for hours in the machine the driving of the resting nuclei into the light hemisphere may be a further cause of abnormality. Since the yolk is thereby deprived of nuclei it may be said in this rather far-fetched way to be injured

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PLATE 1

EXPLANATION OF FIGURES

CUMINGIA

- A* Normal egg.
- B* Centrifuged egg; polar body extruded in pigment area.
- C* Centrifuged egg; polar body extruded in oil cap.
- D* Centrifuged egg; polar body extruded in clear zone.
- E* Centrifuged egg; polar body extruded in clear zone.
- F* Centrifuged egg; with oil cap and pigment displaced.
- G* Centrifuged egg; drawn at 2-cell stage, oil in small cell.
- H* Centrifuged egg; drawn at 2-cell stage, oil in large cell.
- I* Centrifuged egg; drawn at 2-cell stage, oil in both cells.
- J* Centrifuged egg; drawn at 4-cell stage derived from *G*.
- K* Centrifuged egg; drawn at 4-cell stage derived from *H*.
- L* Centrifuged egg; drawn at 4-cell stage derived from *I*.

T. H. MORGAN

G. Riccioli, Del.

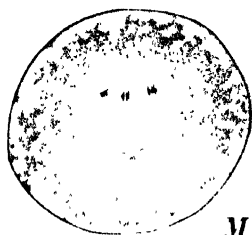
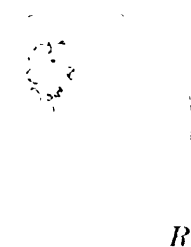
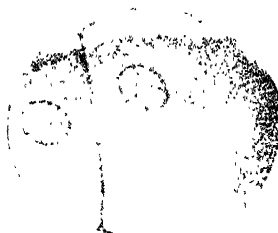
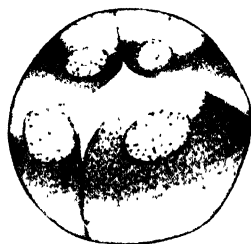
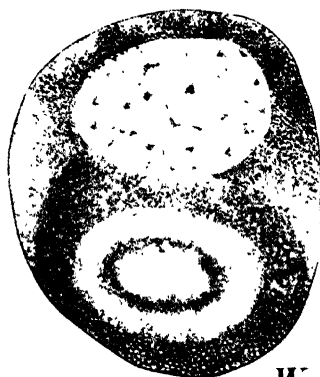
PLATE 2

EXPLANATION OF FIGURES

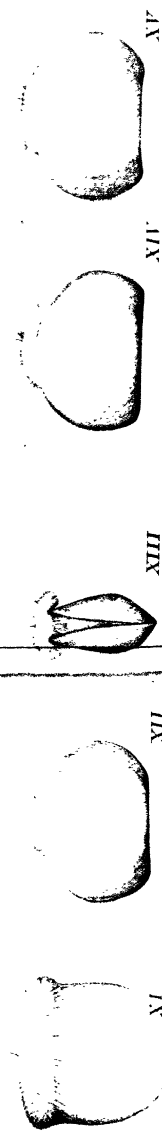
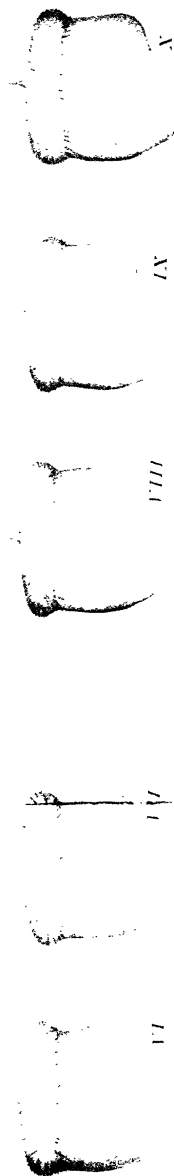
CUMINGIA AND CEREBRATULUS

- M* Section of normal egg just laid.
- N* Centrifuged egg, killed at once.
- O* Centrifuged egg, killed at second polar body stage.
- P* Centrifuged egg, at once, killed at segmentation-spindle stage.
- Q* Centrifuged egg, same.
- R* Centrifuged egg, at two pronuclei stage.
- S* Centrifuged egg, at once, showing yellow centers in asters.
- T* Centrifuged egg, at 2-cell stage.
- U* Centrifuged egg, continuously on water centrifuge
- V* Centrifuged egg, at 4-cell stage.
- W* Cerebratulus egg, centrifuged at germinal vesicle stage
- X* Cerebratulus egg, centrifuged at polar spindle stage.

T. H. MORGAN

*M**N**O**P**Q**R**S**T**U**V**W**X*

G. Riccioli and E. M. Wallace, Del.



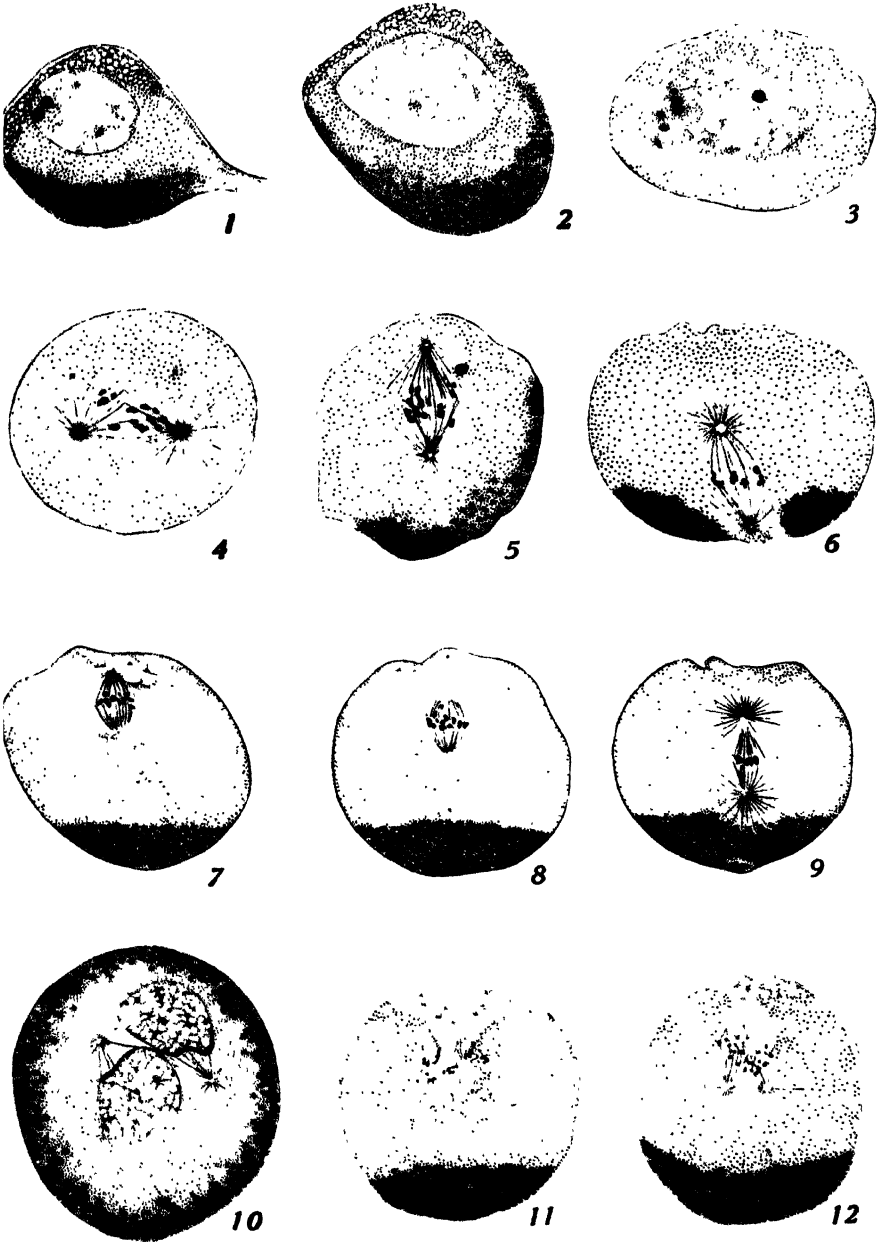
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PLATE 4

EXPLANATION OF FIGURES

CUMINGIA

- 1 Centrifuged ovarian egg with attachment stalk
- 2 Centrifuged ovarian egg with attachment stalk.
- 3 Centrifuged ovarian egg dissolution of germinal vesicle, etc.
- 4 Centrifuged ovarian egg with chromosomes passing to spindle.
- 5 Centrifuged ovarian egg with chromosomes later stage.
- 6 Egg centrifuged at once, killed after half an hour.
- 7 Egg centrifuged when polar spindle was at oil pole.
- 8 Egg centrifuged when polar spindle was at oil pole.
- 9 Egg centrifuged, second polar spindle present, and displaced.
- 10 Normal egg with two pronuclei and segmentation centers.
- 11 Centrifuged 80 turns just before dissolution of pronuclei
- 12 Centrifuged 80 turns just before dissolution of pronuclei.



E. M. Wallace, Del.

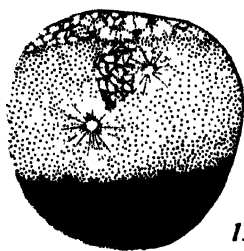
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EXPLANATION OF FIGURES

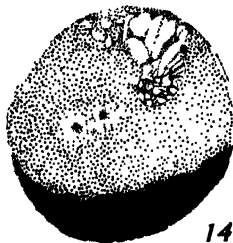
CUMINGIA

- 13 Centrifuged 80 turns at 2 pronuclei stage, killed at once.
- 14 Centrifuged 70 turns at late pronuclei, or even before; continued on water centrifuged until same eggs were ready to go into 2 cells (about twenty minutes on machine). Shows nucleus separated from aster.
- 15 Centrifuged 100 turns at 2 pronuclei stage, beginning 35 minutes after fertilization, continued on water centrifuge until 2 cell (after twenty minutes) This egg polyspermic.
- 16 Ditto not polyspermic.
- 17 Ditto not polyspermic.
- 18 Ditto not polyspermic.
- 19 Ditto not polyspermic.
- 20 Ditto not polyspermic.
- 21 Ditto not polyspermic.
- 22 Ditto not polyspermic.
- 23 Centrifuged at 11-18; killed at 11 30. A M.
- 24 Egg kept all time on water centrifuge; killed just before dissolution of pronucleus.

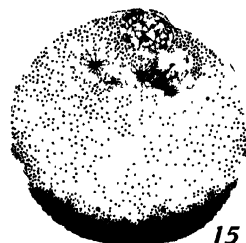
T. H. MORGAN



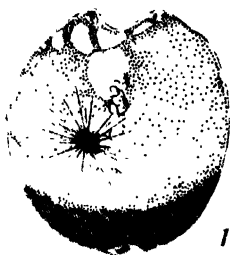
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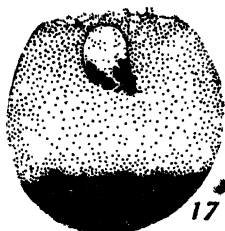
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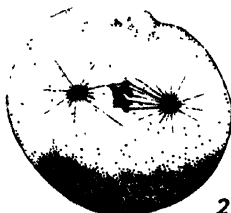
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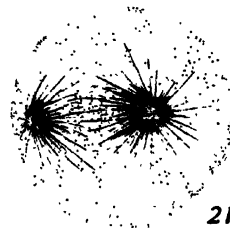
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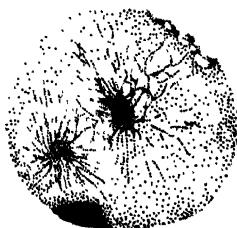
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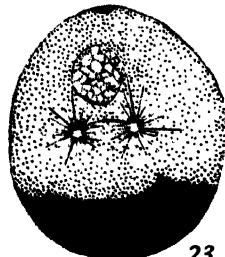
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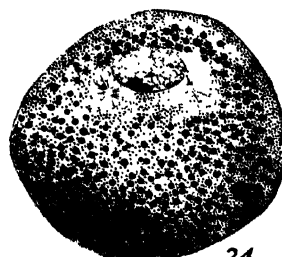
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PLATE 6

EXPLANATION OF FIGURES .

CUMINGIA (25-31) AND CEREBRATULUS (32-35)

- 25 Centrifuged at polar spindle stage; spindle being pushed back by yolk bent fibres.
- 26 Centrifuged 40 turns at dissolution of pronucleus.
- 27 Centrifuged 40 turns at 2-cell stage. The egg had additional male pronuclei or else had a polyaster, but divided into two cells of unequal size.
- 28 Centrifuged while going into 2-cells.
- 29 Centrifuged while going into 2-cells.
- 30 Centrifuged 70 turns at early pronuclei or before, continued on water centrifuge till 2-cell.
- 31 Centrifuged 40 turns as two nuclei reformed. Shows nuclear sap extending shows to oil cap.
- 32 Cerebratulus. Bent segmentation spindles in small piece of egg.
- 33 Centrifuged segmentation spindle.
- 34 Centrifuged segmentation spindle.
- 35 Centrifuged at 2 pronuclei; killed 2-cell.

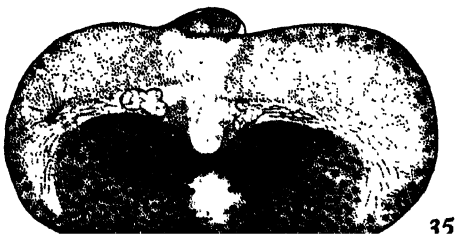
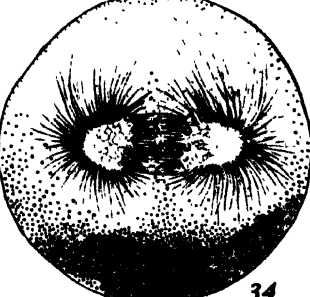
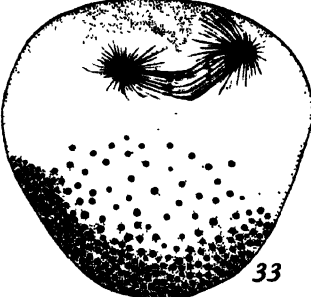
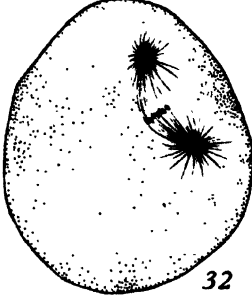
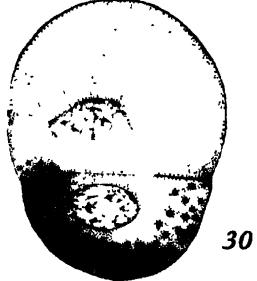
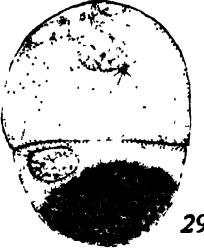
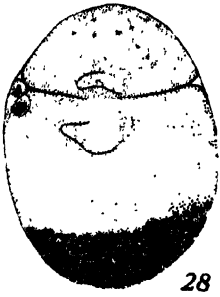
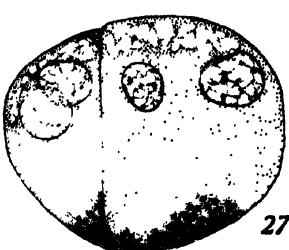
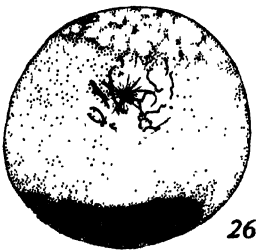
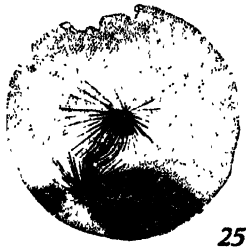
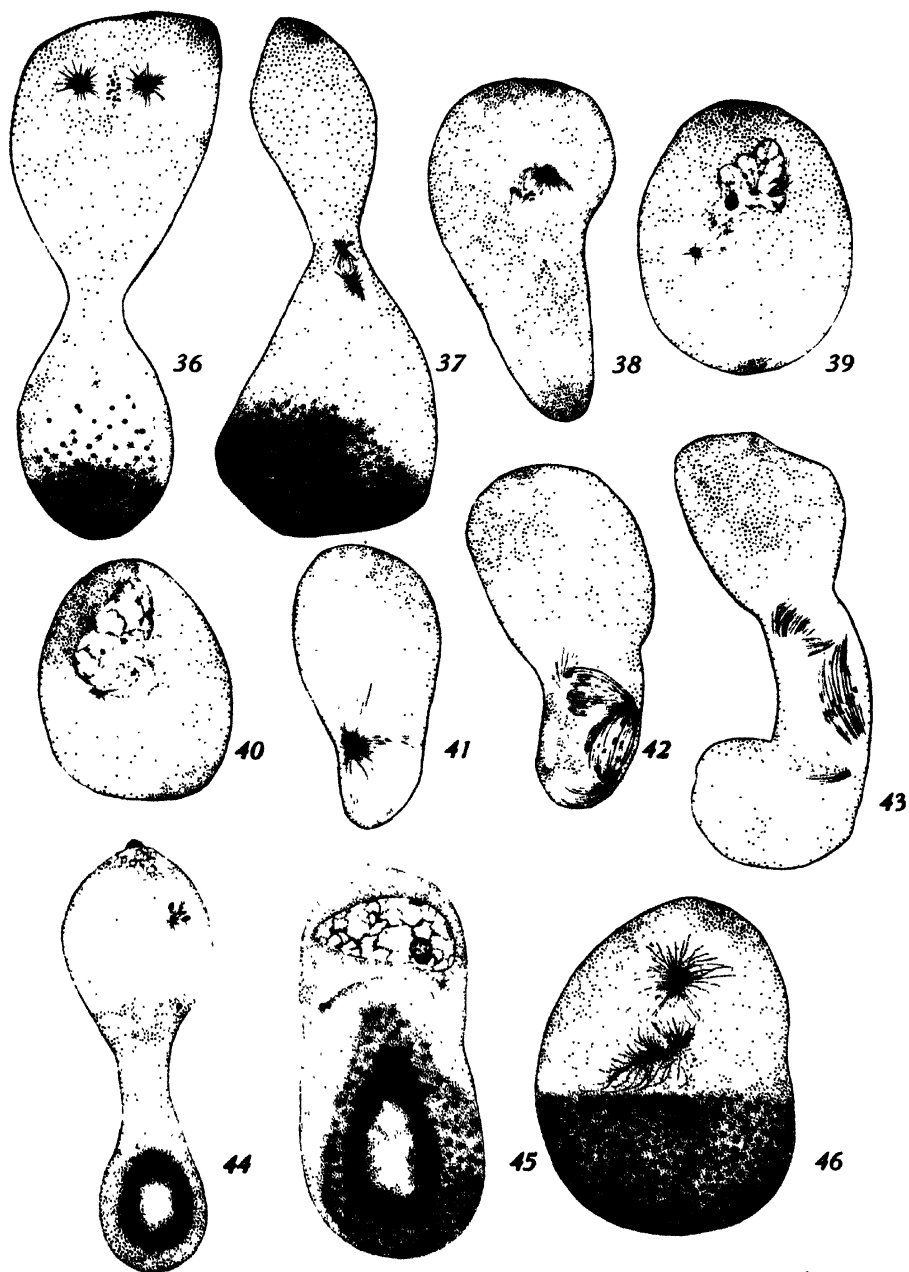


PLATE 7

EXPLANATION OF FIGURES

CEREBRATULUS

- 36 Centrifuged at 2nd polar-spindle stage.
- 37 Centrifuged egg with delayed polar spindles.
- 38 Centrifuged egg segmentation spindle, central rays dislocated
- 39 Centrifuged at 2 pronuclei stage, piece broken off containing pronuclei and their attached asters (polyspermic?)
- 40 Ditto; another section.
- 41 Centrifuged at segmentation spindle stage -one polar aster
- 42 Ditto; bent central spindle.
- 43 Ditto; remainder of last and part of lost aster.
- 44 Centrifuged at 2d polar body stage (at top of egg) shows displaced spindle
- 45 Centrifuged 25 minutes after setting free, showing displaced nucleus and one aster; other represented (?) by the streak near center of egg.
- 46 Distorted segmentation spindle after centrifuging; due to yolk pushing it toward center of egg.



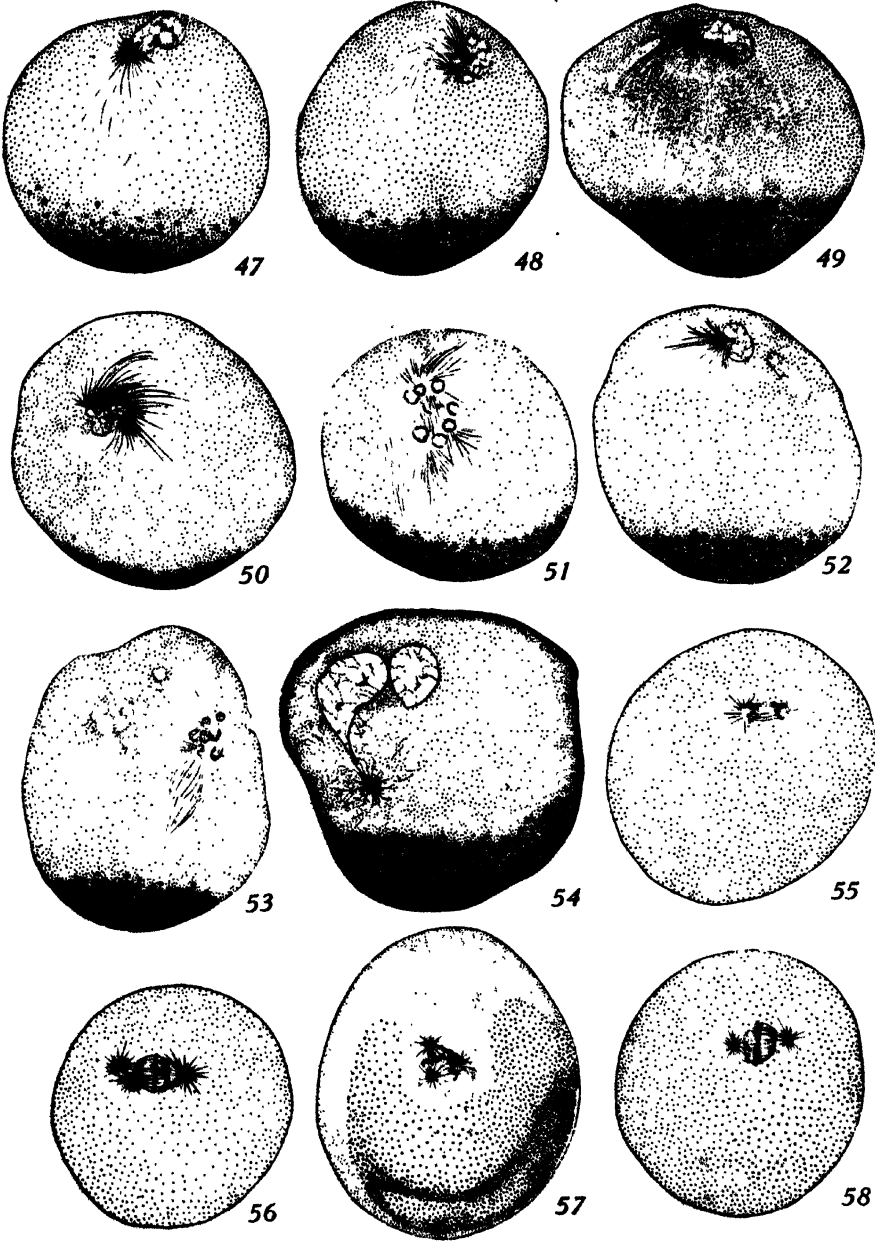
E. M. Wallace, Del.

PLATE 8

EXPLANATION OF FIGURES

CEREBRATULUS

- 47 Centrifuged at 2 pronuclei stage
- 48 Centrifuged at 2 pronuclei stage
- 49 Centrifuged at 2 pronuclei stage.
- 50 Centrifuged at 2 pronuclei stage.
- 51 Centrifuged at 2 pronuclei stage
- 52 Centrifuged at 2 pronuclei stage
- 53 Centrifuged at 2 pronuclei stage.
- 54 Centrifuged at 2 pronuclei stage.
- 55 Delayed polar spindle; chromosomes divided.
- 56 Ditto one polar aster divided.
- 57 Ditto one polar aster divided.
- 58 Delayed polar spindle with large number of chromosomes but with small polar asters.



E. M. Wallace, Del.

VARIATION IN ECHINOID PLUTEI

A STUDY OF VARIATION UNDER LABORATORY CONDITIONS¹

DAVID H. TENNENT

Associate Professor of Biology, Bryn Mawr College

TWENTY-ONE FIGURES

No thoughtful naturalist will question the value of a knowledge of the normal development of a given form as a basis for experimental work. The necessity of a knowledge of the abnormal development is less generally understood.

Our knowledge of the development of invertebrates is of what we term the normal. By normal we mean that occurring under natural conditions and as a part of the course of events in the life history of any organism. Embryos which depart from this type are of little use to us as objects of study or as the basis of figures to be used in illustrating our monographs.

In my observation the fate of an "abnormal" culture is immediate rejection. This treatment is of course what it should be if we desire only an acquaintance with the forms that show the least variation; with possibly the modal life history.

In experimental embryology we need more than this. Our conditions of work are artificial. We must have an exact knowledge of development under these conditions.

¹ The observations upon which this paper is based were made at the United States Fisheries Laboratory at Beaufort, North Carolina, during the summer of 1908. I am indebted to the Hon. George M. Bowers, Commissioner of Fisheries, for permission to work in the laboratory and to Mr. Henry D. Aller, Director of the laboratory, for the excellent facilities for work placed at my disposal. In the determination of the constants I have been assisted by Esther M. Tennent, who has made an independent calculation of all of the results obtained.

In laboratory fertilizations the treatment of eggs and spermatozoa is from the first unnatural. The methods that we adopt are those that will enable us to imitate the natural sequence of events most closely. The successful culture is the one with the greatest number of embryos like those occurring in conditions of nature. All this is admirable, for its purpose.

Suppose, however, that we wish to go a little further, that we desire to study the effect of a change in our artificial normal conditions. We then find ourselves puzzled in determining between the effect caused by the new element that we have introduced into the experiment and the appearance that may be the result of laboratory manipulation.

In 1907 I began an investigation on Cross Fertilization in Echinoids. From my earliest experience as a student in a marine laboratory I had been familiar with the earlier development of most of the forms with which I desired to work.

The cross fertilizations were successfully made. I had no difficulty in seeing that a profound modification in structure had taken place, but from the first I found myself hampered by a lack of exact knowledge of the variation occurring in laboratory cultures.

The observations described in this paper were the result of my attempt to supply myself with the needed information. This information gained at that time has since been of much use in a continuation of the study of cross fertilization.

MATERIAL AND METHODS

The statistics given in this paper are based upon a study of the eggs and embryos of the sea-urchin, *Toxopneustes variegatus*. The breeding season of this echinoid extends throughout the summer, being at its height during June and July.²

² During the three summers preceding 1908 and again in 1908 I noticed that the gonads of sea urchins taken after a night of full moonlight were empty, while those obtained a week later gave an abundance of eggs and spermatozoa. The first exception to the rule was noticed in July, 1908, when after two nights of bright moonlight the supply of *Toxopneustes* obtained was in exceedingly good condition. No observations as to the habits of the sea urchins could be made for the reason that they were obtained from water in which the bottom could not be seen.

Especial care was taken in getting the sea-urchins to the laboratory in the best possible condition. When brought in they were used at once.

Each individual was washed in fresh water, cut horizontally through the test, and the aboral portion, to which the gonads remain attached, placed upside down on a glass plate. In ripe individuals the eggs are at once extruded through the genital pores. Only individuals were used from which the eggs were extruded freely, individuals in which as MacBride has expressed it, "this organ," i.e., the ovary, "at a touch dissolved into eggs."

The eggs were then transferred to sterilized sea water, washed once, and fertilized by the addition of sperm that had been obtained by the same method of extrusion and mixed with sterilized sea water. After the eggs had settled, the supernatant water was poured off and the eggs washed several times in sterilized sea water to get rid of unnecessary sperm.

The percentage of fertilizations was high, practically every egg developing. When the swimming blastulae gathered at the surface of the water, between six and seven hours after fertilization, they were removed and transferred to finger-bowls filled with sterilized sea water, which were then loosely covered with glass plates.

So far as possible absolute cleanliness was observed. The sea water that I have described as "sterilized" was obtained at high tide or at considerable distance from shore. It was brought in

A simple solution of the matter was found during a visit to Tortugas in 1909, when I again worked with *Toxopneustes*. At Boca Grande, where the collecting was done, the sea-urchins were picked up by hand in water sufficiently shallow for wading. During the period of full moon, in two months, *Toxopneustes* was found in abundance, almost in masses, on parts of the bottom that were not covered with vegetation. Such individuals were found to have spawned, while the scattered specimens, obtained from the feeding grounds, gave reproductive elements in abundance.

If we had been collecting here by means of a dredge, as at Beaufort, it seems probable that the greater number of individuals taken at this time would have been devoid of eggs.

There is some evidence, then, that when these animals are ready to spawn they gather more or less closely together and that this movement is correlated with a definite time of month.

in glass vessels, heated in an enameled container to 65° C. filtered through paper and cooled. All dishes and utensils of whatever description were thoroughly boiled prior to being used. A dish once used was boiled before being used again.

That there was an actual value in these precautions was demonstrated by the experience of other workers in the laboratory who were using less particular methods and were unable to keep *Toxopneustes plutei* alive.

The plutei were kept in the finger-bowls, in some cases, as will be noted later, being fed from a diatom culture after the third day, in others kept until the seventh day without the addition of food. The loss of water by evaporation was restored by the addition of sterilized rain water day by day.

By this method I was enabled to carry on a considerable number, the number varying with different series, through their metamorphosis, to the adult condition.

By means of the precautions taken I was able to make fertilizations and to keep the plutei alive throughout August, when by ordinary care in previous years the cultures were ruined by bacteria.

For work of the kind with which this paper deals, we need some standard method of treatment; a method which should be followed by every worker in the field. It is only by using uniform methods that we can hope to obtain results that may be correlated.

The elimination of every possible extraneous influence is desirable. No matter how great the care taken, there are sufficient circumstances over which we have no control. We should adopt the simplest possible method; one which may be applied with equal facility to straight fertilizations or to cross fertilizations.

When the embryos were withdrawn for measurement the contents of the dish were thoroughly stirred up, a portion of the water dipped out and the embryos in this portion thrown down by the addition of fresh water. Sufficient alcohol to preserve the embryos was then added and the measurements made as soon as possible. By this method distortion in form was avoided. Care must be taken that the alcohol be free from acid.

The measurements were made with an ocular micrometer scale in a Zeiss compensating ocular no. 4 with objective AA. The plutei were placed under a large cover and the slides gone over with the aid of a mechanical stage.

All of the arithmetical calculations in this paper are in these micrometer scale units. With the lenses used each eye piece unit is equivalent to .019 mm.

Four measurements were made on each pluteus, namely, from the posterior end of the body to the end of the right anal arm; from the posterior end of the body to the end of the left anal arm;

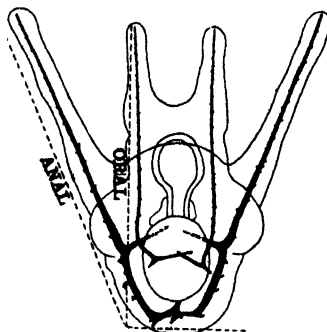


Fig. A

from the posterior end of the body to the end of the right oral arm, and from the posterior end of the body to the end of the left oral arm. The measurements in each case being that of the combined body and arm length. (Fig. A.)

I have selected the skeletal rods as the basis of measurement. In experimental work on echinoid hybrids various investigators have made use of different characters. These characters have been:

1. Form of larvae.
2. Skeleton.
3. Number of chromatophores.
4. Pigment content of chromatophores.
5. Arrangement of chromatophores.
6. Number of primary mesenchyme cells.
7. Size of larvae.

Of these characters I believe that only observations on the skeleton are of practical value. The skeleton in the larvae of each species is of perfectly definite, *i.e.*, a specific form. The species selected for crossing should be those whose plutei have characteristic skeletons.

The chromatophores are amoeboid in nature. I have frequently seen them move out upon the surface of the body and off into the surrounding medium. The arrangement is not specific. The pigment content, although different in various forms, is an exceedingly difficult character to handle, the shades in color from dark red, almost a brown, into bright red, rendering the use of a color chart imperative.

The number of primary mesenchyme cells is a character which may possibly be of value in a study of blastulae, but cannot be made use of in the examination of older larvae.

The size of the larvae is extremely variable even in eggs from the same individual.

The skeleton, on the other hand, is a structure which offers many advantages as a basis for comparisons. It may be readily seen and measured, the normal variations in a given species may be determined, and it is a structure which shows at once the influence of a cross if the cross has been made between properly selected species.

The material upon which this paper is based consists of twenty-four series of eggs, nine of which were studied in detail and measured.

The paper may be conveniently divided into three sections.

Section 1. A statistical consideration of variation,

Section 2. Potential egg variations.

Section 3. Discussion.

1. A STATISTICAL CONSIDERATION OF VARIATION IN ECHINOID PLUTEI

In this section nine series of embryos will be considered, each series derived from the eggs of a different female fertilized by sperm from a different male. We are dealing then with the

progeny of nine females and nine males. In the series I-V observations were made on the second, third, fourth and fifth day of development. For each lot and for each day 100 plutei were measured.

In Series VI observations were made on the third, fourth and fifth day, the plutei being fed after the third day. Here again 100 plutei were measured for each day.

In Series VII measurements on 61 plutei were made on the fourth day.

In Series VIII measurements were made on 100 plutei on the sixth day.

In Series IX measurements were made on 100 plutei on the sixth day.

We find that the variations fall into three classes:

1. Fluctuating variations.
2. Defects.
3. Multiplicities.

The fluctuating variations are those of length and correlation and are grouped more or less symmetrically about a mean. Figs. 1-7 show normal individuals from the end of the first to the end of the seventh day of development. These figures were drawn from living plutei which were selected as individuals that would develop through metamorphosis under favorable conditions, experience having shown that such individuals could be identified at the beginning of the second day. Inspection of these figures will show that the skeleton in each arm consists of a straight rod, slightly roughened by prickles or thorn-like processes. The oral rods are connected with the anal cross bars by a short rod as shown in fig. 4. (See page 666.)

The defects consist in the absence of a skeletal rod or of an arm, or of a deformation of part of the body. Figs. 8, 9, 10, 18, 19, 20.

The multiplicities consist in the presence of more than the normal number of skeletal rods or in more than the usual number of arms. Figs. 11, 12, 13, 14, 16, 17.

An examination of the Tables of Constants, of the Graphic Representation of Variation and of the Correlation Tables, will give any one who is interested in the subject from the standpoint

of an investigator in this field, a full account of the fluctuating variation.

In the text I shall refer only to points of a somewhat general interest which are brought out by the examination of the results. I take only the right anal arm measurement, *i.e.*, the measurement from the posterior end of the body to the tip of the right anal arm.

Series I. The mean, 14.11, is the lowest of any of the series at this age. A diminution in length down through the 4th day is seen. On the 5th day there is an increase. The variation increases through the 4th day and is less on the 5th. Variation in correlation; oral arms on 3d and 4th day longer than anal arms.

Series II. Right anal mean 2d day 14.86. Steady increase during the period studied. 14.86, 17.79, 20.16, 22.54.

Variation: great; rises and falls. Many perfect forms.

Series III. Right anal mean 2d day 16.76. Little change until the 5th day when a noticeable increase began. 16.76, 16.55, 16.96, 20.16.

Variation: high. Highest on the 5th day. Many perfect forms.

Series IV. Right anal mean 2d day 15.86. Steady diminution through each day 15.86, 15.62, 13.65, 12.33.

Variation: relatively low and consistently uniform. A weak series.

Series V. Right anal mean 2d day 17.26. Increase to 4th day, then diminution, then increase, 17.26, 17.57, 16.50, 18.07.

Variation: a gradual lessening.

Series VI. Right anal mean, 3d day, 23.18. Slight increase on the 4th day, decrease on the 5th day. 23.18, 23.48, 23.00.

Variation increases with age; highest on the 5th day; many large individuals.

Series VII. Right anal mean, 4th day, 26.88. Variation high.

Series VIII. Right anal mean, 6th day, 23.12. Variation relatively high.

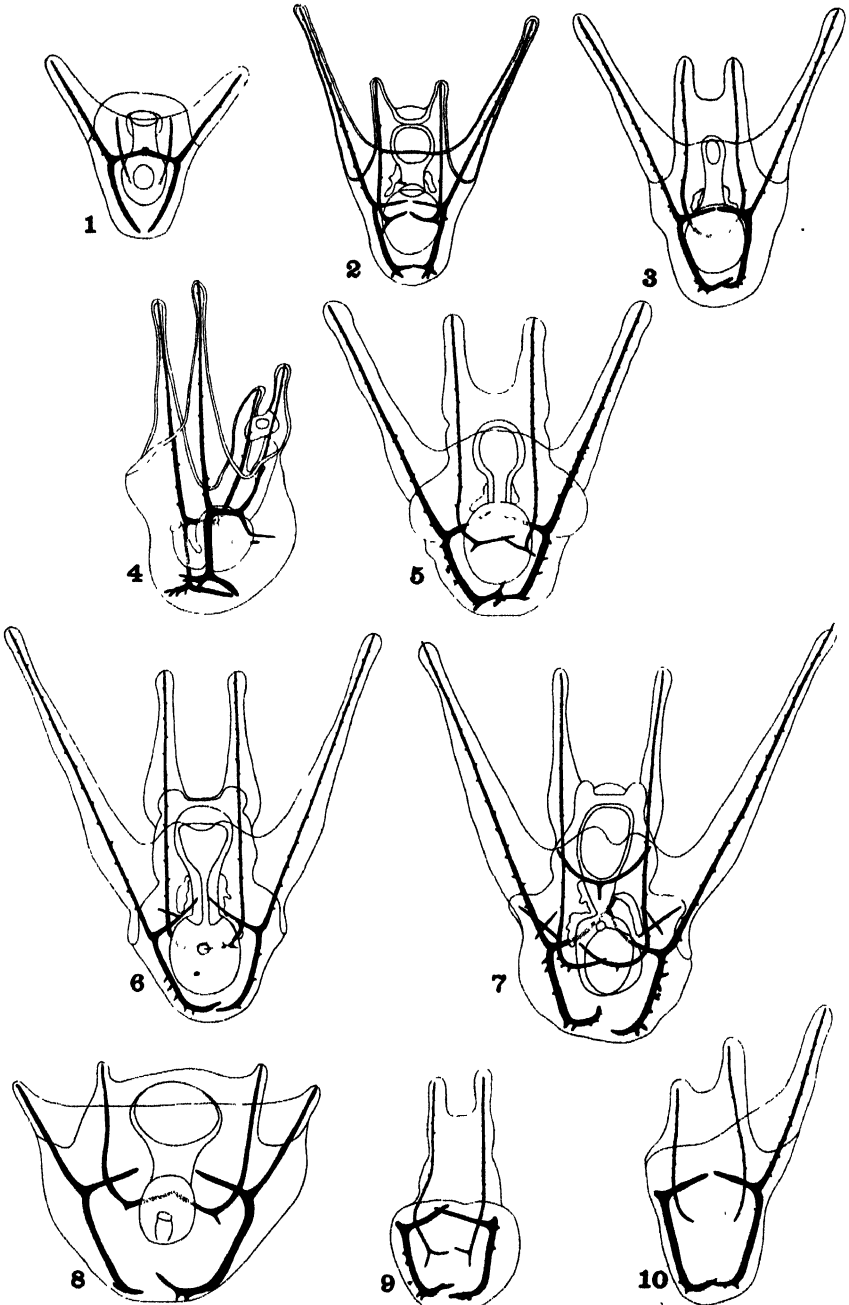
Series IX. Right anal mean, 6th day, 28.81. Variation relatively high.

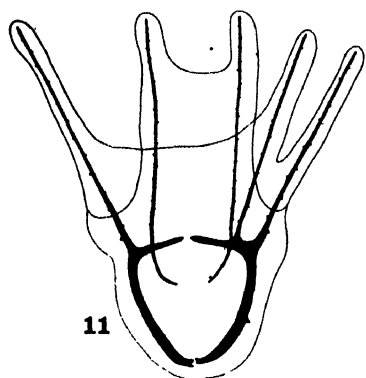
For the combined series I-V, the mean for each group of 500 individuals of the same age was determined and the other constants calculated from these.

EXPLANATION OF FIGURES

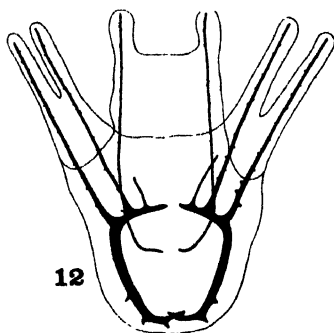
The figures are all drawn from camera lucida sketches and made from living *Toxopneustes plutei*.

1. Ventral view. 24 hrs.
2. Dorsal view. 48 hrs.
3. Ventral view. 60 hrs.
4. Side view. 72 hrs.
5. Dorsal view. 5th day.
6. Ventral view. 6th day.
7. Dorsal view. 7th day.
8. Abnormal pluteus. 8th day.
9. Anal arms lacking. 3d day.
10. One left anal arm. 3d day.
11. Multiple left anal arm. 4th day.
12. Multiple right and left anal arms. 4th day.
13. Multiple oral and anal skeletal rods (one side only shown). 4th day.
14. Multiple right anal skeletal rods. 5th day.
15. Slightly cleft pre-oral region. 4th day.
16. Median anal arm. 5th day.
17. Cleft left anal arm. 4th day.
18. No arms. 4th day.
19. Auricularian type. 3d day.
20. No arms. Rods passing around body. 6th day.

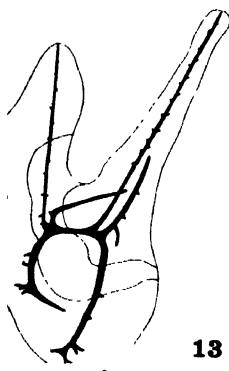




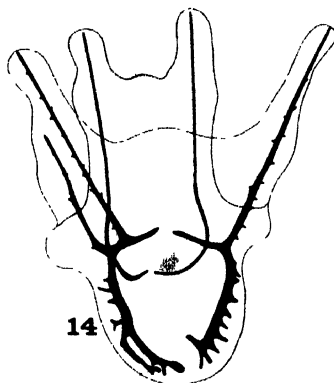
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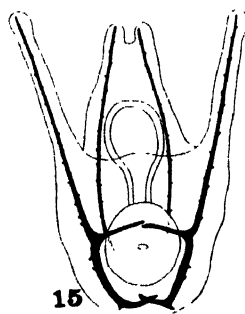
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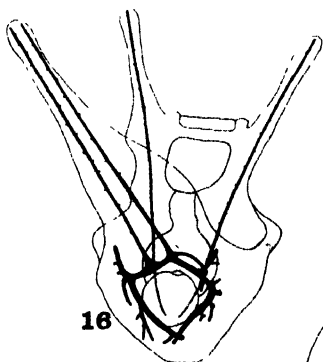
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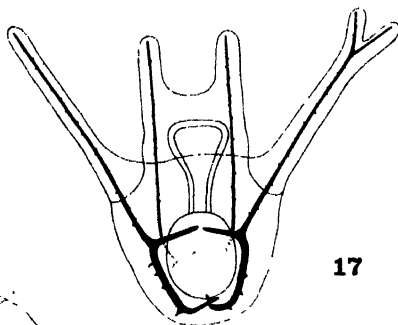
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Series I-V. Right anal mean, 2d day, 15.77. Increase on 3d day; decrease on 4th day; increase on 5th day, 15.77, 16.07, 15.80, 17.51. Variation increases with age.

It will be noticed that Series II and IV differ from the others in their general behavior. Series II showing a steady growth through the period studied and Series IV showing a regular decrease in size.

Series I, III and V, and the general population, represented by the combined Series I-V, agree in a different behavior, all indicating the 4th day as a period of least change, followed by a period of growth. Series VI, the fed series, indicates somewhat the same behavior, with a slight drop on the 5th day.

It is of interest, in this connection, to notice that the plutei in Series III which I checked off as individuals which would have gone through their metamorphosis, show the same behavior. I include a table of means of these individuals.

TABLE 1 *Selected individuals from Series III*

	ANAL		ORAL	
	R.	L.	R.	L.
2 days...	18.54	18.54	13.27	13.45
3 days.	20.00	19.88	16.58	17.54
4 days.	20.81	20.81	17.59	17.54
5 days...	24.54	24.40	20.48	20.54

TABLE 2 *Constants, Series I. Temperature at fertilization 28° C.
Temperature during development 28°-29°*

AGE	STRUCTURE	MEAN	STANDARD DEVIATION	COEFFICIENT OF VARIATION	NUMBER
2 Days	Right Oral	13.11 ± .09	1.34 ± .06	10.28 ± .49	100
	Left Oral	13.11 ± .08	1.32 ± .05	10.10 ± .48	100
	Right Anal	14.11 ± .10	1.56 ± .07	11.11 ± .53	100
	Left Anal	14.09 ± .10	1.55 ± .07	11.04 ± .53	100
3 days	Right Oral	14.36 ± .08	1.30 ± .06	9.05 ± .43	100
	Left Oral	14.36 ± .09	1.34 ± .06	9.36 ± .45	100
	Right Anal	12.81 ± .17	2.53 ± .12	19.79 ± .98	100
	Left Anal	12.72 ± .16	2.51 ± .11	19.76 ± .97	100
4 Days	Right Oral	12.92 ± .09	1.38 ± .06	10.70 ± .51	100
	Left Oral	12.90 ± .09	1.36 ± .06	10.54 ± .50	100
	Right Anal	11.75 ± .19	2.87 ± .13	24.49 ± 1.23	100
	Left Anal	11.47 ± .18	2.81 ± .13	24.51 ± 1.23	100
5 Days	Right Oral	13.68 ± .08	1.26 ± .06	9.23 ± .44	100
	Left Oral	13.74 ± .09	1.36 ± .06	9.90 ± .47	100
	Right Anal	14.46 ± .09	1.41 ± .06	9.79 ± .47	100
	Left Anal	14.22 ± .11	1.68 ± .08	11.82 ± .57	100

TABLE 3 *Constants, Series II. Temperature at fertilization 28° C.
Temperature during development 27.5°-29°.*

AGE	STRUCTURE	MEAN	STANDARD DEVIATION	COEFFICIENT OF VARIATION	NUMBER
2 Days	Right Oral	11.52 ± .12	1.89 ± .09	16.48 ± .80	100
	Left Oral	11.37 ± .12	1.92 ± .09	16.89 ± .82	100
	Right Anal	14.86 ± .18	2.68 ± .12	18.05 ± .88	100
	Left Anal	14.26 ± .19	2.92 ± .13	20.52 ± 1.01	100
3 Days	Right Oral	15.07 ± .21	3.16 ± .15	21.02 ± 1.04	100
	Left Oral	14.79 ± .20	3.08 ± .14	20.86 ± 1.03	100
	Right Anal	17.79 ± .26	3.95 ± .18	22.21 ± 1.11	100
	Left Anal	17.62 ± .27	4.08 ± .19	23.17 ± 1.16	100
4 Days	Right Oral	16.49 ± .19	2.86 ± .13	17.39 ± .85	100
	Left Oral	16.34 ± .19	2.86 ± .13	17.52 ± .86	100
	Right Anal	20.16 ± .21	3.13 ± .14	15.57 ± .75	100
	Left Anal	19.74 ± .22	3.37 ± .16	17.08 ± .83	100
5 Days	Right Oral	18.27 ± .22	3.37 ± .16	18.45 ± .90	100
	Left Oral	18.05 ± .23	3.43 ± .16	19.00 ± .93	100
	Right Anal	22.54 ± .22	3.34 ± .15	14.82 ± .72	100
	Left Anal	22.35 ± .24	3.61 ± .17	16.16 ± .79	100

TABLE 4 Constants, Series III. Temperature at fertilization 27.5° C.
Temperature during development 27.5°-29°

AGE	STRUCTURE	MEAN	STANDARD DEVIATION	COEFFICIENT OF VARIATION	NUMBER
2 Days	Right Oral	11.46 ± .10	1.59 ± .07	13.87 ± .67	100
	Left Oral	11.48 ± .10	1.59 ± .07	13.90 ± .67	100
	Right Anal	16.76 ± .14	2.16 ± .10	12.93 ± .62	100
	Left Anal	16.54 ± .15	2.30 ± .10	13.92 ± .67	100
3 Days	Right Oral	11.82 ± .19	2.94 ± .14	24.93 ± 1.26	100
	Left Oral	11.79 ± .20	3.00 ± .14	25.47 ± 1.29	100
	Right Anal	16.55 ± .21	3.15 ± .15	19.07 ± .94	100
	Left Anal	16.41 ± .21	3.19 ± .15	19.48 ± .96	100
4 Days	Right Oral	12.76 ± .22	3.37 ± .16	26.45 ± 1.34	100
	Left Oral	12.73 ± .22	3.37 ± .16	26.53 ± 1.35	100
	Right Anal	16.96 ± .18	2.77 ± .13	16.37 ± .80	100
	Left Anal	16.89 ± .18	2.77 ± .13	16.40 ± .80	100
5 Days	Right Oral	15.79 ± .29	4.33 ± .20	27.47 ± 1.40	100
	Left Oral	15.81 ± .29	4.36 ± .20	27.57 ± 1.41	100
	Right Anal	20.16 ± .28	4.18 ± .19	20.74 ± 1.03	100
	Left Anal	20.03 ± .28	4.19 ± .19	20.92 ± 1.04	100

TABLE 5 Constants, Series IV. Temperature at fertilization 29° C.
Temperature during development 28°-29°

AGE	STRUCTURE	MEAN	STANDARD DEVIATION	COEFFICIENT OF VARIATION	NUMBER
2 Days	Right Oral	9.47 ± .09	1.47 ± .07	15.54 ± .75	100
	Left Oral	9.49 ± .09	1.47 ± .07	15.58 ± .76	100
	Right Anal	15.86 ± .11	1.65 ± .07	10.43 ± .50	100
	Left Anal	15.79 ± .10	1.56 ± .07	9.89 ± .47	100
3 Days	Right Oral	9.92 ± .08	1.32 ± .06	13.34 ± .64	100
	Left Oral	9.86 ± .08	1.27 ± .06	12.90 ± .62	100
	Right Anal	15.62 ± .14	2.08 ± .09	13.32 ± .64	100
	Left Anal	15.20 ± .13	2.03 ± .09	13.38 ± .64	100
4 Days	Right Oral	9.17 ± .07	1.12 ± .05	12.23 ± .59	100
	Left Oral	9.14 ± .07	1.06 ± .05	11.67 ± .56	100
	Right Anal	13.65 ± .12	1.79 ± .08	13.11 ± .63	100
	Left Anal	13.41 ± .12	1.86 ± .08	13.87 ± .67	100
5 Days	Right Oral	8.56 ± .07	1.08 ± .05	12.72 ± .61	100
	Left Oral	8.54 ± .07	1.12 ± .05	13.18 ± .64	100
	Right Anal	12.33 ± .12	1.80 ± .08	14.59 ± .71	100
	Left Anal	12.25 ± .12	1.85 ± .08	15.11 ± .73	100

TABLE 6 *Constants, Series V. Temperature at fertilization 28.5° C.
Temperature during development 27.5°-28.5°*

AGE	STRUCTURE	MEAN	STANDARD DEVIATION	COEFFICIENT OF VARIATION	NUMBER
2 Days	Right Oral	11.56 ± .10	1.49 ± .07	12.90 ± .62	100
	Left Oral	11.81 ± .09	1.46 ± .06	12.36 ± .59	100
	Right Anal	17.26 ± .19	2.95 ± .14	17.10 ± .83	100
	Left Anal	17.50 ± .19	2.83 ± .13	16.18 ± .79	100
3 Days	Right Oral	12.86 ± .13	2.03 ± .09	15.81 ± .77	100
	Left Oral	12.81 ± .13	2.07 ± .09	16.17 ± .79	100
	Right Anal	17.57 ± .13	2.05 ± .09	11.66 ± .56	100
	Left Anal	17.27 ± .15	2.34 ± .11	13.57 ± .65	100
4 Days	Right Oral	12.70 ± .09	1.47 ± .07	11.59 ± .56	100
	Left Oral	12.49 ± .09	1.40 ± .06	11.23 ± .54	100
	Right Anal	16.50 ± .11	1.64 ± .07	9.97 ± .48	100
	Left Anal	16.40 ± .12	1.82 ± .08	11.10 ± .53	100
5 Days	Right Oral	13.31 ± .09	1.46 ± .06	10.96 ± .52	100
	Left Oral	13.41 ± .09	1.37 ± .06	10.28 ± .49	100
	Right Anal	18.07 ± .13	1.98 ± .09	10.99 ± .53	100
	Left Anal	18.14 ± .13	2.00 ± .09	11.05 ± .53	100

TABLE 7 *Constants, mean of the means, Series I-V*

AGE	STRUCTURE	MEAN	STANDARD DEVIATION	COEFFICIENT OF VARIATION	NUMBER
2 Days	Right Oral	11.42 ± .05	1.95 ± .04	17.08 ± .37	500
	Left Oral	11.45 ± .05	1.95 ± .04	17.04 ± .37	500
	Right Anal	15.77 ± .07	2.55 ± .05	16.18 ± .35	500
	Left Anal	15.64 ± .08	2.65 ± .05	17.00 ± .37	500
3 Days	Right Oral	12.81 ± .08	2.93 ± .06	22.93 ± .51	500
	Left Oral	12.72 ± .08	2.90 ± .06	22.86 ± .51	500
	Right Anal	16.07 ± .10	3.37 ± .07	20.97 ± .46	500
	Left Anal	15.84 ± .10	3.42 ± .07	21.59 ± .48	500
4 Days	Right Oral	12.81 ± .09	3.21 ± .06	25.12 ± .56	500
	Left Oral	12.72 ± .09	3.18 ± .06	24.21 ± .54	500
	Right Anal	15.80 ± .11	3.83 ± .08	24.28 ± .54	500
	Left Anal	15.58 ± .11	3.87 ± .08	24.85 ± .56	500
5 Days	Right Oral	13.92 ± .12	4.16 ± .08	29.91 ± .69	500
	Left Oral	13.91 ± .12	4.13 ± .08	29.75 ± .68	500
	Right Anal	17.51 ± .13	4.61 ± .09	26.36 ± .60	500
	Left Anal	17.40 ± .14	4.67 ± .09	26.89 ± .61	500

TABLE 8 *Constants, Series VI-IX*

AGE	STRUCTURE	MEAN	STANDARD DEVIATION	COEFFICIENT OF VARIATION	NUMBER
VI. Temperature at Fertilization 27.5°. During development 27°-27.5°					
3 Days	Right Oral	18 68 ± 08	1.33 ± 06	7.13 ± 34	100
	Left Oral	18 62 ± 08	1.24 ± 05	6.69 ± 32	100
	Right Anal	23 18 ± 14	2.08 ± 09	8.99 ± 43	100
	Left Anal	23 09 ± 13	2.06 ± 09	8.96 ± 43	100
4 Days	Right Oral	19 26 ± 09	1.40 ± 06	7.28 ± 34	100
	Left Oral	19.31 ± 11	1.65 ± 07	8.56 ± 41	100
	Right Anal	23.48 ± 14	2.16 ± 10	9.19 ± 44	100
	Left Anal	23.57 ± 17	2.54 ± 12	10.80 ± 52	100
5 Days	Right Oral	18 62 ± 10	1.48 ± 07	7.99 ± 38	100
	Left Oral	18.65 ± 09	1.45 ± 06	7.81 ± 37	100
	Right Anal	23 00 ± 15	2.23 ± 10	9.70 ± 46	100
	Left Anal	23 03 ± 14	2.17 ± 10	9.46 ± 45	100
VII. Temperature at fertilization 24°. During development 24°-25°					
4 Days	Right Oral	21.03 ± 30	3.48 ± 21	16.57 ± 1.0 3	61
	Left Oral	20 80 ± 30	3.54 ± 21	17.05 ± 1 0 7	61
	Right Anal	26 88 ± 38	4.40 ± 26	16.40 ± 1 0 2	61
	Left Anal	27.06 ± 38	4.42 ± 27	16.36 ± 1 0 2	61
VIII. Temperature at fertilization 26°. During development 26°-28°					
6 Days	Right Oral	18 35 ± 23	3.47 ± 16	18.95 ± 93	100
	Left Oral	18 52 ± 24	3.58 ± 17	19.33 ± 95	100
	Right Anal	23.12 ± 26	3.93 ± 18	17.01 ± 83	100
	Left Anal	22 93 ± 27	4.12 ± 19	17.99 ± 88	100
IX. Temperature at fertilization 27°. During development 27°-28°					
6 Days	Right Oral	23 76 ± 24	3.64 ± 17	15.33 ± 74	100
	Left Oral	23.69 ± 24	3.59 ± 17	15.15 ± 73	100
	Right Anal	28.81 ± 31	4.60 ± 21	15.97 ± 78	100
	Left Anal	28 60 ± 31	4.69 ± 22	16.41 ± 80	100

Since neither the defects nor the multiplicities appear as such in the tables of constants, although the defects have influenced the size of the mean in every case, a separate treatment of these variations is necessary. I mention these as percentages in the various series studied. *

Series I.

- 2 days. No anal arms (type, fig. 9), 1 per cent.
No left oral arm, 1 per cent.
- 3 days. No anal arms, 8 per cent.
No oral arms, 7 per cent.
- 4 days. No anal arms, 16 per cent.
No oral arms, 28 per cent.
- 5 days. No defects.

Series II.

- 2 days. No anal arms, 1 per cent.
No oral arms, 6 per cent.
No left anal arm (type, fig. 10) 2 per cent.
With both multiple right and left anal arm (type, fig. 12), 3 per cent.
With multiple right anal arm (type, fig. 11), 4 per cent.
With multiple left anal arm, 5 per cent (one with three rods).
- 3 days. No anal arms, 3 per cent.
No oral arms, 4 per cent.
Multiple right anal arm, 2 per cent.
Multiple left anal arm, 2 per cent.
Left anal arm cleft (type, fig. 17), 1 per cent.
Slightly cleft pre-oral region, 5 per cent.
- 4 days. No oral arms, 4 per cent.
Multiple left anal arm, 2 per cent.
Slightly cleft pre-oral region, 4 per cent.
- 5 days. No left anal arm, 1 per cent.
No left oral arm, 1 per cent.
Multiple right anal arm, 1 per cent.
Multiple left anal arm, 2 per cent.
Slightly cleft pre-oral region, 2 per cent.

Series III.

- 2 days. Multiple left anal rods. (type, fig. 14) 2 per cent.
- 3 days. No anal arms, 1 per cent.
No oral arms, 1 per cent.
Multiple right anal rods, 1 per cent.
Uncleft pre-oral region, 1 per cent.
- 4 days. Slightly cleft pre-oral region, 5 per cent.
- 5 days. Slightly cleft pre-oral region, 7 per cent.

Series IV.

- 2 days. Split left anal arm, 1 per cent.
- 5 days. No oral arms, 5 per cent.

Series V.

- 2 days. No right anal arms, 4 per cent.
No left anal arms, 1 per cent.
No oral arms, 3 per cent.
Cleft left anal arm, 1 per cent.
- 3 days. No anal arms, 1 per cent.
No left anal arm, 1 per cent.
- 4 days. No anal arms, 2 per cent.
No oral arms, 3 per cent.

Series VI.

- 3 days. No anal arms, 1 per cent.
- 4 days. One oral arm, 1 per cent.
Multiple anal rods, 2 per cent.
Multiple oral rods, 1 per cent.
- 5 days. Multiple anal rods, 1 per cent.

Series VII (61 plutei).

- 4 days. No anal arm, 1 pluteus.
One anal arm, 1 pluteus.
No oral arms, 4 plutei.

Series VIII.

- No arms. Rods passing around the body (fig. 20), 1 per cent.
- One right anal arm, 4 per cent.
- One left anal arm, 3 per cent.
- Multiple skeletal rods right anal (type, fig. 14), 2 per cent.
- Multiple skeletal rods left anal, 3 per cent.
- Multiple right anal arm, 2 per cent.
- Multiple left anal arm, 2 per cent.
- No oral arms, 6 per cent.
- One right oral arm, 2 per cent.
- One left oral arm, 1 per cent.

Series IX.

- Median anal arm with multiple rods (type, fig. 6), 2 rods, 4 per cent, 3 rods 3 per cent.
- No right anal arm, 5 per cent.
- No left anal arm, 4 per cent.
- Multiple right anal rods (type, fig. 14), 3 per cent.
- Multiple left anal rods (type, fig. 14), 2 per cent.
- Slightly cleft anal ridge, 1 per cent.
- No right oral arm, corresponding left with 2 bars, 2 per cent.
- No left oral arm, corresponding right with 2 bars, 2 per cent.
- Pre-oral region slightly cleft, 1 per cent.
- Pre-oral region uncleft, 1 per cent.

2. POTENTIAL EGG VARIATIONS

The results noted in the preceding section indicated the possible existence of definite type or line variations. Further information on this point seemed desirable and I, therefore, undertook a very simple set of experiments.

Experiment 1. Five females A, B, C, D, and E were selected and the eggs of each placed in a separate aquarium. All of these were fertilized with sperm from a single male, F. The resulting embryos then were AF, BF, CF, DF and EF. Temperature at fertilization 27° C. During development 27°-28°.

Two things were noted as a result of this experiment. First, the time of beginning of cleavage and the rate of cleavage are nearly constant in a given lot of eggs. Lot CF began its separation into two cells in 39 minutes. Lot DF showed no constriction until 43 minutes after impregnation. Second, a variation; larvae of an auricularian type in lot AF. On the third day when a count of one hundred embryos was made 13 per cent were found to be of this type, the body elongated and with neither oral nor anal arms. A skeleton was present.

Experiment 2. Five females A, B, C, D, and E were selected and the eggs of each divided into two portions, A₁-E₁ and A₂-E₂, each portion being placed in a separate aquarium. Lots A₁-E₁ were fertilized with sperm from male F. Lots A₂-E₂ were fertilized with sperm from male G. Temperature at fertilization 27° C. During development 27°-28°. Lots A₁F and A₂G showed the same variation, a divided condition of either the right anal or of the left anal arm. (Type, fig. 17.) No other noticeable multiplicity or defect occurred.

Experiment 3. Five females, A, B, C, D and E were selected and the eggs of each divided into five portions A₁-A₅ through E₁-E₅, and each lot placed in a separate aquarium.

Lots A₁-E₁ were fertilized with sperm from male F
Lots A₂-E₂ were fertilized with sperm from male G
Lots A₃-E₃ were fertilized with sperm from male H
Lots A₄-E₄ were fertilized with sperm from male K
Lots A₅-E₅ were fertilized with sperm from male M

Temperature at fertilization 28°. During development 27°-28°. After the 3rd day all of the A series, *i.e.*, A₁F, A₂G, A₃H, A₄K, A₅M were characterized by the presence of a high percentage of larvae which under favorable conditions would have completed their metamorphosis. With a slight deviation either way, of a hundred plutei counted from each of the five lots, 21 per cent showed these characters.

Series B was weak from the first. At the end of the 3d day all of this lot with the exception of B₄M were dead.

Series C was weak. C₄M living longest.

Series D showed 15 per cent normal larvae of the type described for series A; 4 per cent showed the uncleft condition of the pre-oral region. This variation occurring throughout the series.

Series E. Six per cent normal larvae (type described for A). Three per cent with a short additional skeletal rod in one anal arm.

To sum up, these series of eggs show characteristic line variations:

Experiment 1. A. Auricularian type.

Experiment 2. A. Divided condition of an anal arm.

Experiment 3. A. Great number of normal larvae.

B. Weakness.

C. Weakness.

D. Strength. Uncleft condition of pre-oral region.

E. Good percentage of normal larvae. Extra skeletal rod in one anal arm.

These variations, it will be noted, are peculiar to the eggs and make their appearance irrespective of the kind of sperm used. The only noticeable influence of the sperm is in lots B₄M and C₄M, where the sperm seemed to confer a greater vitality.

3. DISCUSSION

In the field in which these observations lie is the work of Steinbrück ('02) and Vernon ('95, '98 and '00), Vernon's two later papers being based upon his development of the subject in his first. Vernon has also made use of his observations in his "Variation in Animals and Plants."

His work of 1895 is especially valuable in that he shows the effect of environment on what I have termed in this paper "fluctuating variations."

It is evident to the reader that the objective point in Vernon's work is not the same as in mine. He has "sought to establish that this variation may be considerably increased by the operation

of changes in the environment during development," while I have tried to determine the amount of variation and the direction of variation in distinct lines kept, so far as possible, in uniform environments.

Vernon's results and my own are in some respects complementary and I hope, at no distant date, to make a detailed comparison of his numerical results with mine.

Steinbrück's work deals particularly with the variations that I have termed multiplicities. It is interesting to see that *Strongylocentrotus*, with which these investigators, Vernon and Steinbrück, have worked shows some of the multiplicities that are shown by its relative, *Toxopneustes*, which has been the species under my observation.

In section 1 of this paper the differences in variability and the amount of variation from day to day have been shown. The only comment that I wish to make is that in the various series, as will be seen by an inspection of the graphic representations, there is not a symmetrical grouping about the mean. A closer approach to symmetry is seen in the combined Series I-V and an approximation to the theoretical curve is made by the individuals that I have checked in Series III as "normal."

My observations on the individual series agree with Vernon's anal arm measurements (a measurement of the anal arm length and not including the body length).

In Section 1 it has further been shown that there are characteristic type or line variations. These are:

Series I. Defects in arms. Long oral arms.

Series II. Multiple right and left anal arms. Slightly cleft pre-oral region.

Series III. Slightly cleft pre-oral region.

Series IV. Weakness.

Series V. Defects in arms.

Series VI. Multiple rods.

Series VII. No marked characteristics.

Series VIII. Extreme variation as to multiplicities and defects.

Series IX. Single median anal arm containing two or more bars.

The work of the second section shows as type characteristics:

Series I. Auricularian type.

Series II. Divided condition of anal arm.

Series III. High percentage of normal larvae.

Series IV. Weakness.

Series V. Uncleft condition of pre-oral region.

Series VI. Multiple rods in anal arms.

The appearance of these characters suggests germinal variations, but as I have pointed out before, they are of the nature of line or type variations. We are not, to be sure, dealing with pure lines in Johannsen's sense, nevertheless it is evident that the early development of these embryos shows a mean, which is not the mean of the general population of echinoid plutei, and each series or line exhibits its characteristic variations.

The ability of the egg to impress its stamp on the embryo, is due, I believe, to its condition at the time of fertilization. I do not refer to a relative maturity of egg and spermatozoan, a conception that I cannot quite grasp, if the two are sufficiently mature to unite and develop, but to condition in the sense of tendency toward dominance. This condition is not fixed, but may be changed. (Tennent, 1910.)

Viewed in this light, the mature egg, as it comes from the ovary and is passed into the sea water, carries with it a specific and an individual tendency. If fertilization be delayed and the egg subjected to factors changing its condition the chances of a dominance of paternal characters are greater.

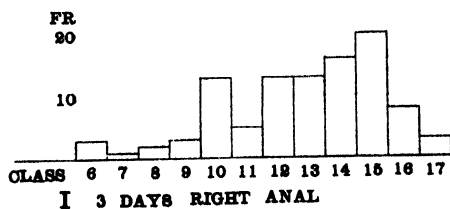
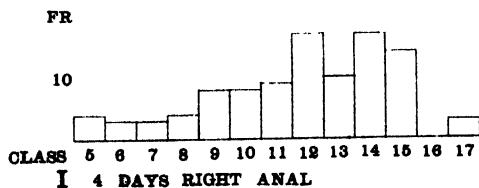
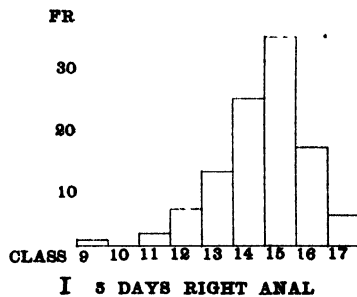
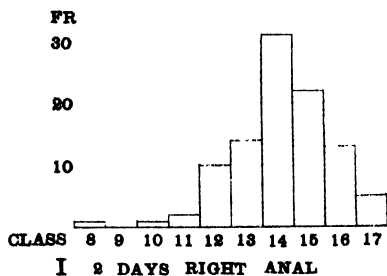
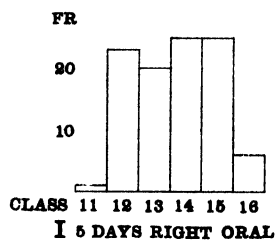
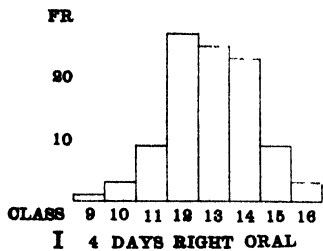
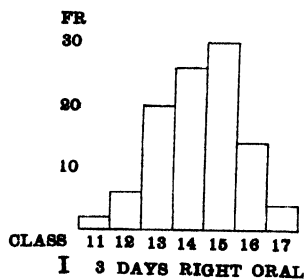
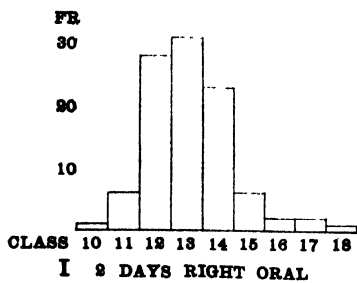
In conclusion I must urge most strongly that for any cross that is made with such organisms as we are discussing, a control be kept which will enable the investigator to determine the type variations occurring in the individuals with which he is working.

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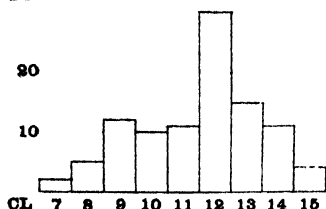
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GRAPHIC REPRESENTATION OF VARIATION

For each of the Series I-V and for the combined Series I-V the classes and frequency of the right oral and the right anal lengths are shown by the method of rectangles.



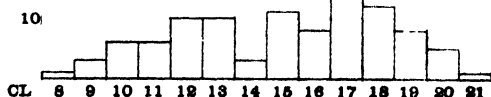
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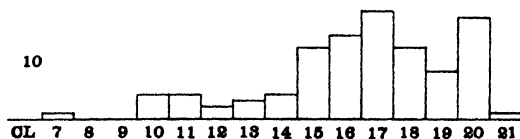
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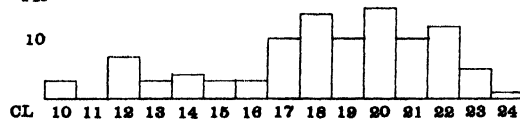
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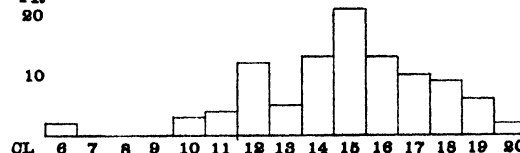
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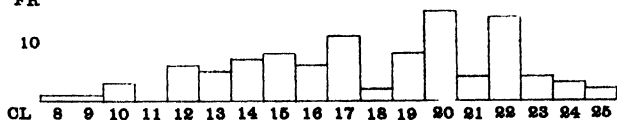
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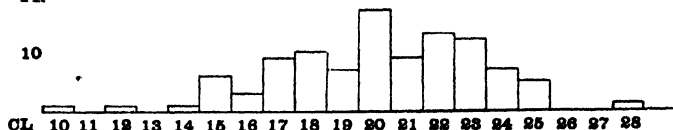
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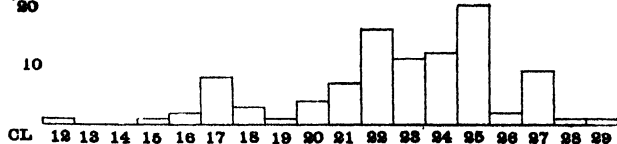
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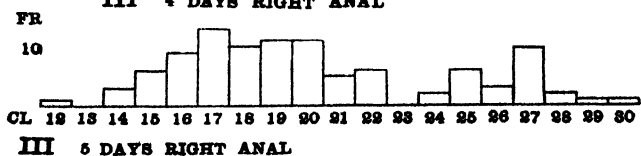
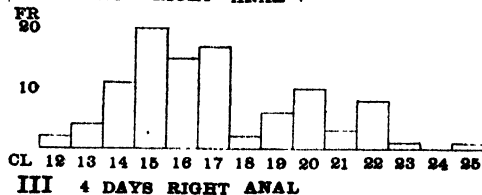
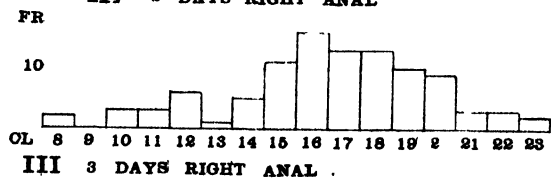
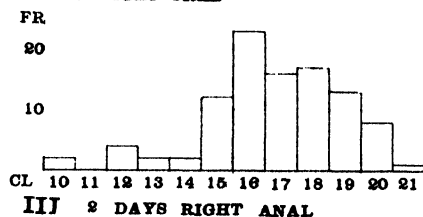
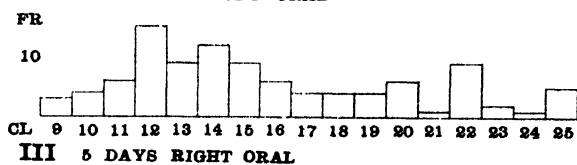
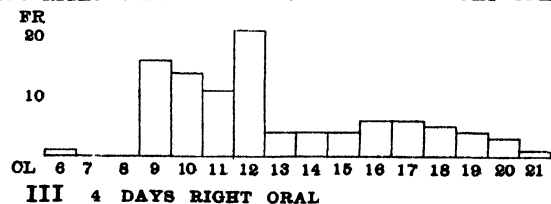
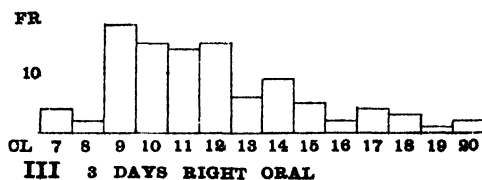
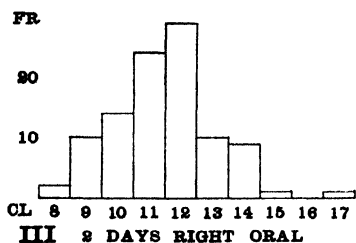
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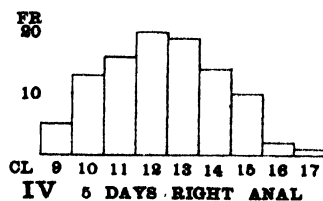
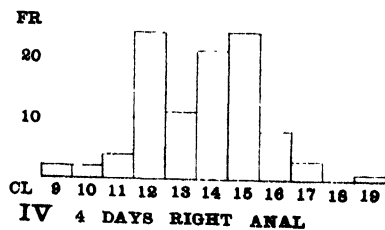
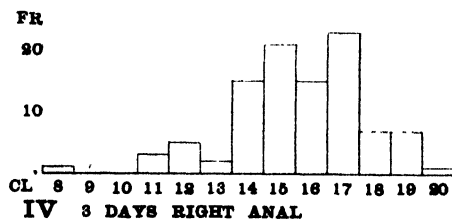
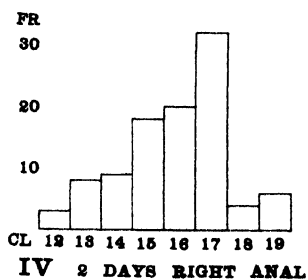
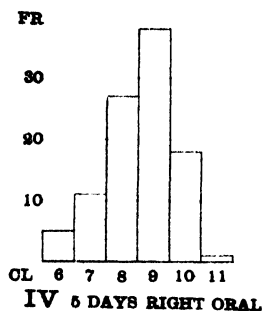
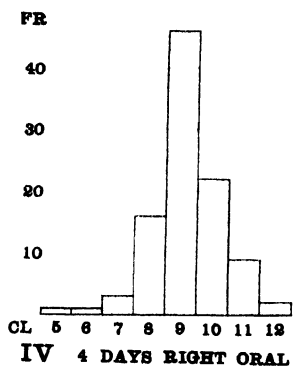
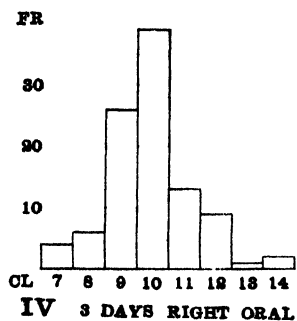
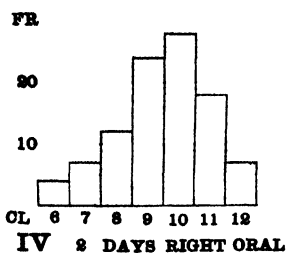
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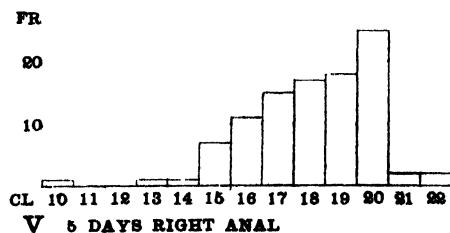
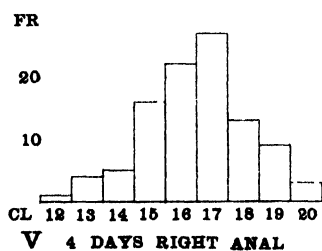
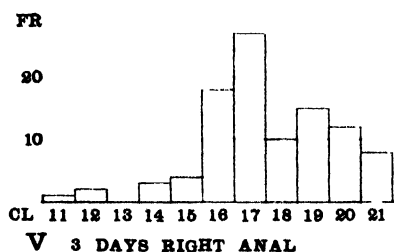
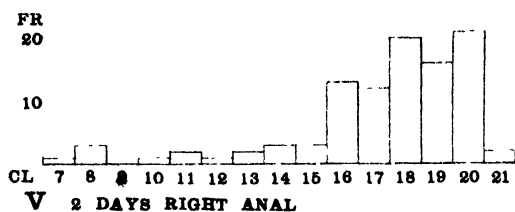
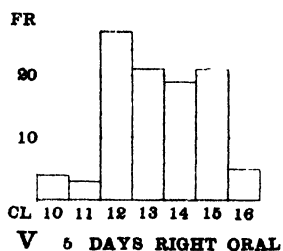
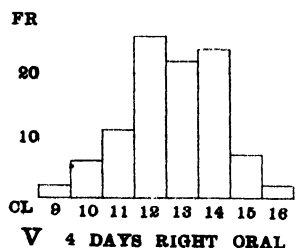
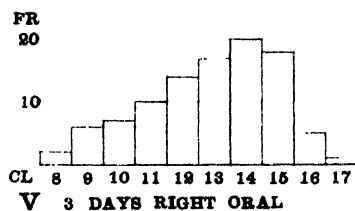
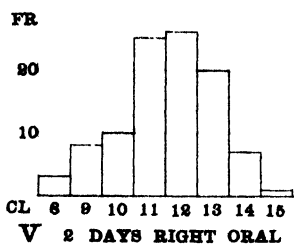
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II 9 DAYS RIGHT ORAL







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180

110

100

90

80

70

60

50

40

30

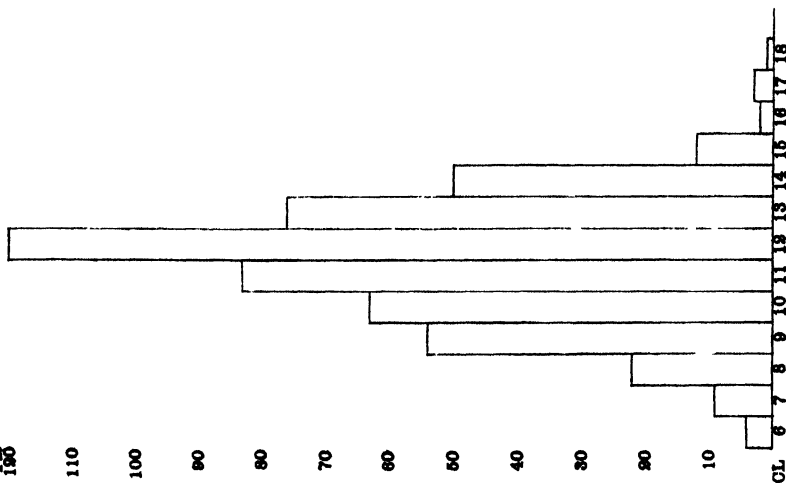
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OL

6 7 8 9 10 11 12 13 14 15 16 17 18

I-V 3 DAYS RIGHT ORAL



FR

80

70

60

50

40

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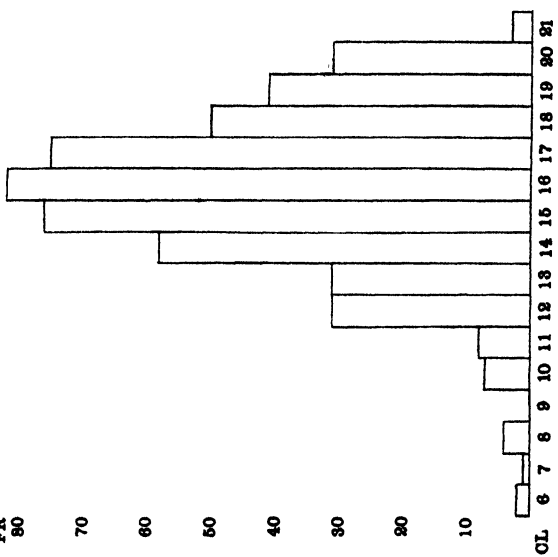
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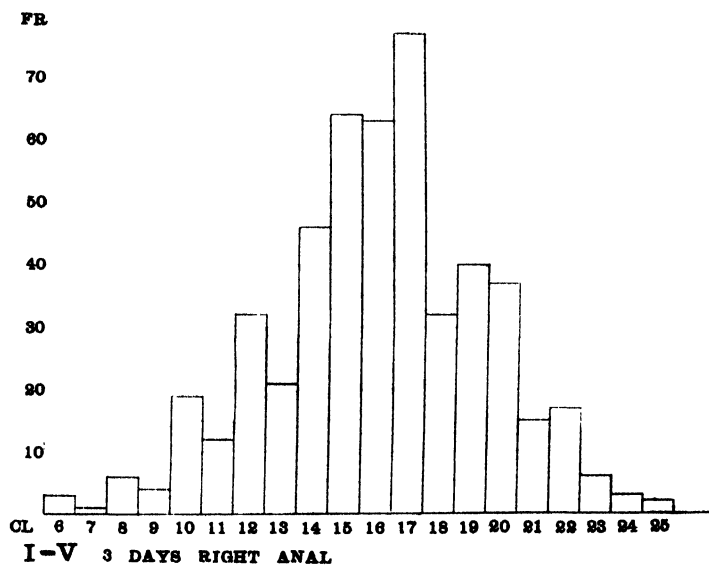
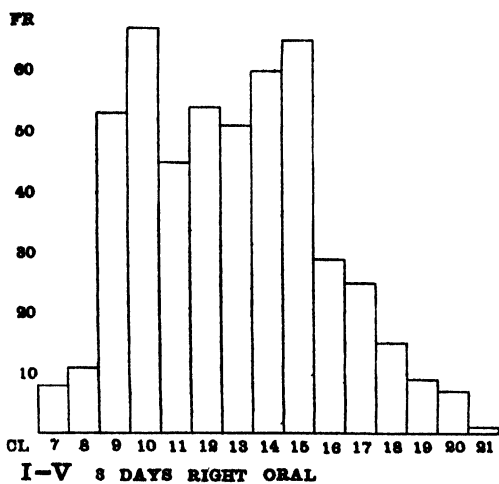
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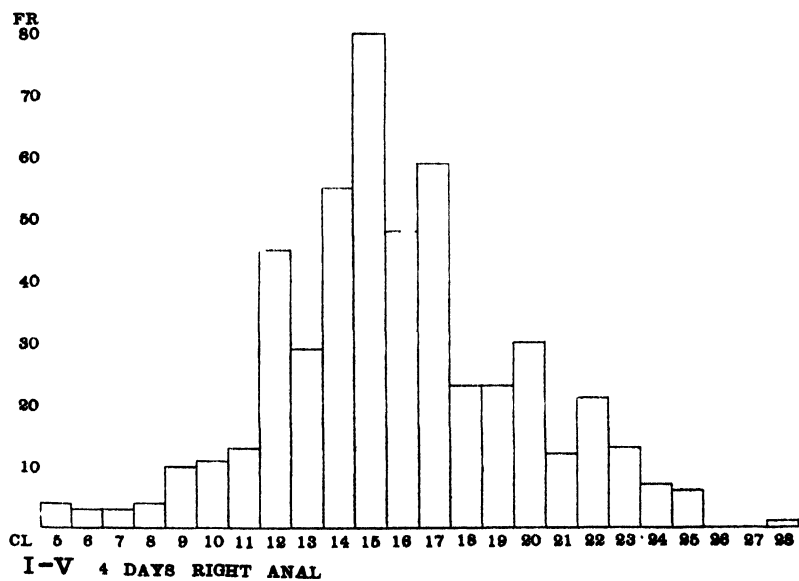
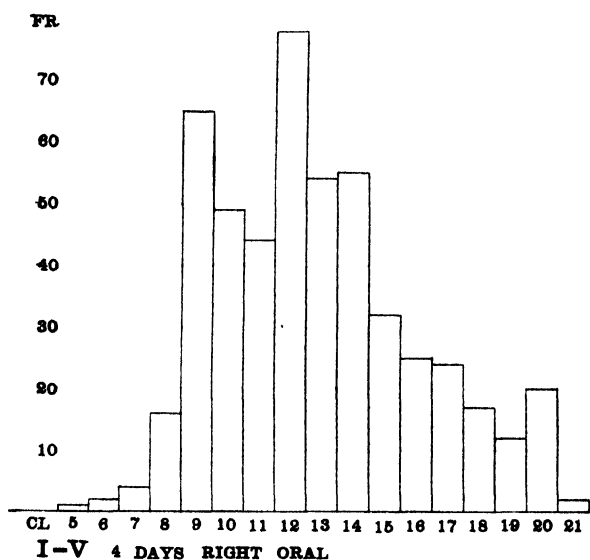
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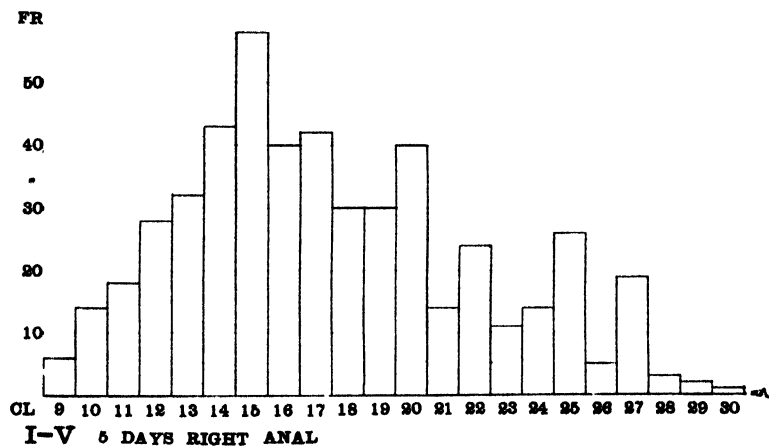
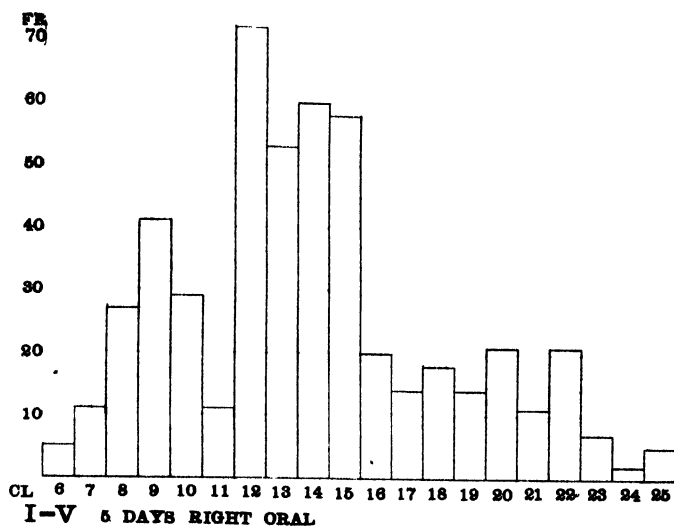
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I-V 3 DAYS RIGHT ANAL









APPENDIX

CORRELATION TABLES

THE CORRELATION OF RIGHT ORAL AND RIGHT ANAL LENGTHS

<i>I. Right Oral Arm, 2 Days</i>											<i>I. Left Oral Arm, 2 Days</i>										
	10	11	12	13	14	15	16	17	18			10	11	12	13	14	15	16	17	18	
<i>I. Right Anal Arm, 2 Days</i>	8		1						1	8			1							1	
9									0	9										0	
10			1						1	10			1							1	
11		2							2	11		2								2	
12		2	2	4	2	1			11	12		2	2	4	2	1				11	
13		1	7	4	2				14	13		1	7	4	2					14	
14	1	1	5	12	10			2	31	14	1	1	5	12	11			2		32	
15			9	8	4				1	22	15			7	10	4			1	22	
16			1	3	4	4	1		13	16			1	3	4	3	1			12	
17			2		1	1	1		5	17			2	1		1	1			5	
	1	6	28	31	23	6	2	2	1			1	6	26	34	23	5	2	2	1	

		<i>I. Right Oral Arm, 3 Days</i>									<i>I. Left Oral Arm, 3 Days</i>						
<i>I. Right Anal Arm, 3 Days</i>		11	12	13	14	15	16	17	<i>I. Left Anal Arm, 3 Days</i>		11	12	13	14	15	16	17
	6		2	1				3		6		2		1			3
	7			1				1		7			1				1
	8			2				2		8			1	1			2
	9		2		1			3		9		3				1	4
	10	2	1	6	3		1	13		10	1	2	2	4	1	1	11
	11			1		1	1	5		11	1		1	3	2		7
	12			3	3	6	1	13		12			3	3	6	1	13
	13			2	6	5		13		13			3	3	8	1	15
	14				4	10	1	16		14			2	4	7	2	17
	15		1	1	7	5	6	20		15		1		6	4	5	17
	16				2	1	4	8		16		1		1		4	6
	17					3		3		17					3	1	4
		2	6	17	26	31	14	4			2	9	13	26	31	15	4

I. Right Oral Arm, 4 Days

	9	10	11	12	13	14	15	16	
<i>I. Right Anal Arm, 4 Days</i>									
5		1	1	1		1			4
6		1	1	1					3
7				2		1			3
8			1		1	1	1		4
9			1		2	5			8
10	1		1	3	1	1	1		8
11				5	2	2			9
12		1	1	2	7	5	1		17
13				2	3	1	2	2	10
14			1	7	5	3	1		17
15			2	3	4	2	3		14
16									0
17				1		1		1	3
	1	3	9	27	25	23	9	3	

I. Left Oral Arm, 4 Days

	9	10	11	12	13	14	15	16	
<i>I. Left Anal Arm, 4 Days</i>									
5		1	1	1		1			4
6		1	1	1					3
7				2	1	1			4
8			1	1	1	1			4
9			1		3	3			7
10		1	1	4	4	2	1		13
11			2	5	2	1	1		11
12		1		3	3	5	1	1	14
13				4	1	1	4	1	11
14			1	4	4	3	2		14
15			1	4	5		3		13
16					1				1
17						1			1
	0	4	9	29	25	19	12	2	

I. Right Oral Arm, 5 Days

	11	12	13	14	15	16	
<i>I. Right Anal Arm, 5 Days</i>							
9		1					1
10							0
11		1	1				2
12		2	3		1		6
13		5	2	4	1		12
14	1	7	4	5	7		24
15		5	7	11	10	1	34
16		1	2	4	4	5	16
17		1	1	1	2		5
	1	23	20	25	25	6	

I. Left Oral Arm, 5 Days

	11	12	13	14	15	16	17	
<i>I. Left Anal Arm, 5 Days</i>								
9		1						1
10					1			1
11			2	1		1		4
12		6	3	1	1			11
13		4	2	5	1			12
14		7	6	5	6			24
15	1	3	3	8	9	1		25
16		1		3	6	4	1	15
17	1	1	1	2		1		6
18						1		1
	2	23	17	25	24	8	1	

II. Right Oral Arm, 2 Days

	7	8	9	10	11	12	13	14	15	
6		1				1			2	
7									0	
8									0	
9									0	
10		1	1					1	0	
11	1	2					1		4	
12			2	4	1	4			12	
13		1	1		1	2			5	
14			4	2	1	2	4		13	
15	1		3	3	4	9	1		21	
16					3	5	1	4	13	
17					1	3	4	1	10	
18			1	1		2	1	3	9	
19						2	2	2	6	
20							1		2	
	2	5	12	10	11	30	15	11	4	

II. Left Oral Arm, 2 Days

	7	8	9	10	11	12	13	14	15	
5	1									1
6										0
7										0
8				1		1				2
9					1					1
10		1	2	2	1	3				9
11		1		2			1			4
12			5	3	1	1			1	11
13		2	2		1	1	2			8
14		1	4	1	1	4	2			13
15	1		1	1	4	9	1	1		18
16				1	1	3	1	4	1	11
17					2	1	2	3		8
18			1			2	2	2		7
19						1	2	1		4
20						1		1	1	3
	2	5	15	11	12	27	13	12	3	

II. Right Oral Arm, 3 Days

	8	9	10	11	12	13	14	15	16	17	18	19	20	21	
8					1										1
9				1											1
10	1	1				1									3
11															0
12			1	1	1	1		1		1					6
13				1	1	1		1		1					5
14			1			4	1		1						7
15			2	1		3						1	1		8
16			1			3		2							6
17					2	2	1	2	3	1					11
18									1	1					2
19					1	2		1		2	1	1			8
20							3	3	3	2	3		1		15
21										3	1				4
22										4	4	5	1		14
23								1		1	1	1			4
24											1		1	1	3
25											1				2
	1	3	6	6	10	10	3	11	8	16	12	8	5	1	

II. Left Oral Arm, 3 Days

	8	9	10	11	12	13	14	15	16	17	18	19	20	21
7					1									1
8														0
9			1											1
10	1	1			1									3
11				1										1
12		1	1	1	1		1	2						7
13			1	1	2					1				5
14		1		2	1	2								6
15			2	1	1	3			1		1		1	10
16		1		1	2	2		3	1	1				9
17					2	1		2	1					6
18										2				2
19					1	2		1		1	1	1		7
20						1	1	3	1	4	3			13
21					1					4	1			6
22							1	1		4	5	3	1	15
23								1		1	1			3
24										1			1	3
25											2			2
	1	4	5	7	11	11	3	13	4	19	14	4	3	1

II. Right Oral Arm, 4 Days

	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	
10					1											1
11																0
12				1												1
13					1											0
14					1											1
15				1	1	1	1			1	1					6
16							1	2								3
17				2		1	1		2	3						9
18	1				1			1	4	1						8
19									1	2	3		1			7
20								1	5	5	4	2				17
21										1	4	2	2			9
22											6	3	2	2		13
23										1		4	1	6		12
24												1	1	4	1	7
25														5		5
26																0
27																0
28													1			1
	1	0	0	4	4	2	3	4	12	14	18	12	8	17	1	

II. Left Oral Arm, 4 Days

	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
10					1										1
11															0
12				1	1						1		1		4
13															0
14					1										1
15				1	1	1	1		2	1					7
16							1	2		1					4
17				1		1	1		1	2					6
18	1					2		1	5		1				10
19						1			1	2	2	1	1		8
20									5	4	4	2		1	16
21										1	3	2	2		8
22								1	1		5	3	1	2	13
23												3	3	4	10
24												1		6	7
25													1	3	4
26															0
27												1			1
28															0
	1	0	0	3	4	5	3	4	15	11	16	13	9	16	

II. Right Oral Arm, 5 Days

	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	
<i>II. Right Anal Arm, 5 Days</i>																
12	1															1
13																0
14			1													1
15			1													1
16					1		1									2
17	1		2	2	1	1		1								8
18	1				2											3
19								1								1
20			1	1				1	1							4
21			1			1		2	1		2					7
22						1	1	4	4	4	2					16
23							1	1	3	3	2	1				11
24									3	2	4	1	2			12
25			1					2	1	4	5	6	1			20
26											2					2
27										1	1	3	3	1		9
28												1				1
29														1		1
	3	0	7	3	4	3	3	10	14	10	15	10	12	5	1	

II. Left Oral Arm, 5 Days

	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
9				1												1
10																0
11																0
12		1														1
13																0
14				1												1
15				2												2
16						1	1									2
17				4		1	1		1							7
18		1				2										3
19									1							1
20				1	1				1	1						4
21							1		2	1		2				6
22	1						2	1	4	3	4	2				17
23								1	2	3	3	2				11
24										3	2	3				13
25				1						1	1	8	4	4		19
26													1			1
27												1	1	3	3	9
28														1		1
29															1	1
	1	2	0	9	2	4	5	2	11	12	10	18	8	11	4	1

III. Right Oral Arm, 2 Days

	8	9	10	11	12	13	14	15	16	17	
10		2									2
11											0
12		1	1	2							4
13				1	1						2
14		1	1								2
15	1	2	1	4	3	1					12
16	1	1	2	8	7	2	2				23
17		1	1	5	4	1	3			1	16
18			5	2	6	3	1				17
19		1	3	1	5	1	1	1			13
20		1		1	2	2	2				8
21					1						1
	2	10	14	24	29	10	9	1	0	1	

III. Left Oral Arm, 2 Days

	8	9	10	11	12	13	14	15	16	17	
10		2									2
11			1	2							3
12	1	1	1								3
13				1	1						2
14		1			2						3
15		2	1	4	3	2					12
16	1	1	1	7	7	3	1				21
17		1	3	6	5	1	2	1		1	20
18			4	4	4	2	1				15
19		1	2		4	1	1	1			10
20		1		1	1	3	2				8
21					1						1
	2	10	13	25	28	12	7	2	0	1	

III. Right Oral Arm, 3 Days

	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
8	1						1								2
9															0
10	1		2												3
11			2	1											3
12			2	1	2	1									6
13		1													1
14			2			3									5
15			2	2	2	3		1	1						11
16	1	1	6	3	1		1	3							16
17				2	3	4	2	1		1					13
18	1			2	2	3	1	3			1				13
19			1	4	2	1	1		1						10
20			1		2			1	2		1	2			9
21									1	1				1	3
22											1	1	1		3
23											1			1	2
	4	2	18	15	14	15	6	9	5	2	4	3	1	2	

III. Left Oral Arm, 3 Days

	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
8	1														1
9															0
10	1		1	1											3
11			3	2	1										6
12			2	2	1										5
13		1	2				1								4
14			1		2	1		1							5
15			1	1	5	2		1							10
16	1	1	6	2	1		1	3	1						16
17				2	2	3	2			1					10
18	1			2	2	3	1	3			1				13
19				4	1	1	1	1			1				9
20			1	1	3			1	2		1	1			10
21									1					1	3
22											1		1	2	4
23											1				1
	4	2	17	17	18	10	6	10	4	1	5	2	1	3	

III. Right Oral Arm, 4 Days

	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	
<i>III. Right Anal Arm, 4 Days</i>				1	1												2
12				1	1	2											4
13				4	3		4										11
14				3	3	5	8	1									20
15				4	2	3	5							1			15
16				3	3		2	2	3	2	1						17
17	1						1			1							2
18						1	1	1			2						6
19					1	1	1		1	1	3	4	1				10
20													1	1	1		3
21														1		1	8
22												2	3	1			1
23														1			0
24																1	1
25																	
	1	0	0	16	14	11	21	4	4	4	6	6	5	4	3	1	

III. Left Oral Arm, 4 Days

	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	
<i>III. Left Anal Arm, 4 Days</i>				1	1												2
12				1	1	2											4
13				4	3	1	4										12
14				4	4	4	8	1									21
15				4	2	2	4							1			13
16				2	3	1	4	2	3	3							19
17	1									1							1
18																	5
19					1		1	1			2						10
20									2	1	2	4	1				4
21													1	1	2		7
22												2	2	2		1	1
23														1			0
24																1	1
25																	
	1	0	0	16	15	10	21	4	5	5	4	6	4	5	3	1	

III. Right Oral Arm, 5 Days

	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	
12		1																1
13																		0
14			1	1	1													3
15	1		2	1	1		1											6
16			3	3	1	2												9
17	1	1		5	1	5												13
18	1	2		3	1	1	2											10
19				1	2	2	3	2	1									11
20					2	1	2	1	2	1	2							11
21							1	2		1		1						5
22				1		1			1	1	2							6
23																		0
24												1			1			2
25								1				4	1					6
26										1				2				3
27														7		1	2	10
28																	2	2
29																1	1	1
30															1			1
	3	4	6	15	9	12	9	6	4	4	4	6	1	9	2	1	5	

III. Right Anal Arm, 5 Days

III. Left Oral Arm, 5 Days

	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
12		1	1														2
13																	0
14			1	1	1												3
15	1		1	2	2		1										7
16			3	1	2												8
17	1	1		5	2	4											13
18	1	2		3	1		2										9
19				1	2	2	3	3									11
20					2	1	2	1	1	3	1						11
21				1			1	1			2	1					6
22						1			1	1	2						5
23																	0
24												2			1		3
25									1			4		1			6
26									1					2	1		4
27													1	3	1	1	9
28																	1
29																1	1
30															1		1
	3	4	6	14	12	10	9	5	4	4	5	7	1	6	4	1	5

IV. Right Oral Arm, 2 Days

	6	7	8	9	10	11	12
12	1	1	1				3
13	1		3	3	1		8
14	2	2	1	3		1	9
15			5	2	7	2	18
16		2	1	2	8	5	20
17		2	1	9	11	8	32
18					1	1	2
19				5		1	6
	4	7	12	24	28	18	7

IV. Right Anal Arm, 2 Days

IV. Left Oral Arm, 2 Days

	6	7	8	9	10	11	12
12		1	1				2
13	1		2	4	1		8
14	2	2	1	1	2	1	9
15			6	4	6	3	21
16	1	2	2	3	10	5	1
17		2		7	10	6	2
18					1	1	2
19				3	1		1
	4	7	12	22	31	16	8

IV. Left Anal Arm, 2 Days

IV. Right Oral Arm, 3 Days

	6	7	8	9	10	11	12	13	14
8				1					1
9									0
10									0
11				2		1			3
12		1		2	2				5
13					1	1			2
14		2	1	6	6				15
15			3	5	7	3	3		21
16			1	3	7	2	1	1	15
17		1		6	9	3	3	1	23
18			1		4	2			7
19				1	3	1	2		7
20								1	1
	4	6	26	39	13	9	1	2	

IV. Right Anal Arm, 3 Days

IV. Left Oral Arm, 3 Days

	7	8	9	10	11	12	13	14
8								0
9								0
10			1		1			2
11			3		1			4
12	1		1	4				6
13		1		3				4
14	2	1	8	3	1		1	16
15		2	8	9	2	1	1	23
16		2	3	8	2	1		16
17	1		4	8	4	4		21
18				3	1			4
19					1	1		2
20					1		1	2
	4	6	28	38	14	7	2	1

IV. Left Anal Arm, 3 Days

IV. Right Oral Arm, 4 Days

	5	6	7	8	9	10	11	12	
8									0
9		1			1				2
10				1	1				2
11					1	2	1		4
12	1		3	3	8	7	2		24
13				6	4			1	11
14				2	12	6	1		21
15				4	14	3	3		24
16					4	3	1		8
17						1	1	1	3
18									0
19					1				1
	1	1	3	16	46	22	9	2	

IV. Left Oral Arm, 4 Days

	5	6	7	8	9	10	11	12	
7	1								1
8									0
9		1			1				2
10			1	1					2
11					4	3			7
12			2	4	6	8	1		21
13				7	8	2			17
14				1	10	5	1		17
15				3	12	5	4		24
16					3	3	1		7
17								1	1
18									0
19					1				1
	1	1	3	16	45	26	7	1	

IV. Right Oral Arm, 5 Days

	6	7	8	9	10	11	
9	1	2	1	1			5
10	1		4	6	2		13
11	1	3	6	4	2		16
12	2	4	5	5	4		20
13		1	7	8	2	1	19
14		1	2	7	4		14
15			2	6	2		10
16				1	1		2
17					1		1
	5	11	27	38	18	1	

IV. Left Oral Arm, 5 Days

	6	7	8	9	10	11	
8	1						1
9		2	1	1	1		5
10	1	1	5	6	1		14
11	2	3	5	5	1		16
12	2	5	5	6	3		21
13			6	6	2	1	15
14			2	7	3	1	13
15			2	7	4		13
16				1	1		2
	6	11	26	39	16	2	

V. *Right Oral Arm, 2 Days*

	8	9	10	11	12	13	14	15
V. <i>Right Anal Arm, 2 Days.</i>								
7				1				1
8	1		1	1				3
9								0
10	1							1
11				2				2
12			1					1
13			1	1				2
14		2		1				3
15		1		1	1			3
16		2	2	3	3	2	1	13
17		2	2	3	4	1		12
18	1			7	7	5		20
19		1	1	3	6	4	1	16
20			2	2	5	8	3	1 21
21							2	2
	3	8	10	25	26	20	7	1

V. *Left Oral Arm, 2 Days*

	8	9	10	11	12	13	14	15
V. <i>Left Anal Arm, 2 Days.</i>								
6					1			1
7								0
8			1					1
9				1				1
10	1							1
11				1				1
12		1	1					2
13								0
14			2	2	1			5
15		1	2					3
16	1	1	1	2	5	1	1	12
17		1		5	6			12
18		2		4	9	4	1	20
19			1	3	4	6	1	15
20				3	7	4	5	2 21
21					1		3	4
22						1		1
	2	6	8	21	34	16	11	2

V. Right Oral Arm, 3 Days

	8	9	10	11	12	13	14	15	16	17
<i>V. Right Anal Arm, 3 Days</i>										
11			1							1
12			1		1					2
13										0
14	1		1	1						3
15		3			1					4
16	1	1	2	3	2	6	3			18
17			3	1	6	3	6	8		27
18				2	1	2	3	2		10
19			1	2	2	2	5	3		15
20				1	1	3	3	2	2	12
21						1		3	3	1
	2	6	7	10	14	17	20	18	5	1

V. Left Oral Arm, 3 Days

	8	9	10	11	12	13	14	15	16	17
<i>V. Left Anal Arm, 3 Days</i>										
9					1					1
10		1								1
11	1	1								2
12		1								1
13										0
14	1		1	2						4
15		2		3		1				6
16			2	3	3	4	1	2		15
17			3	2	7	1	7	7		27
18		1		1	3	3	2	2		12
19			1	1	1		5	3	1	12
20					2	2	5	5	1	1
21								1		2
22										0
23									1	1
	2	6	7	12	17	11	20	20	3	2

V. Right Oral Arm, 4 Days

	9	10	11	12	13	14	15	16
V. Right Anal Arm, 4 Days								
12	1							1
13			1	3				4
14		4		1				5
15	1	1	4	7	1	2		16
16		1	5	3	9	4		22
17				10	3	10	4	27
18			1	1	6	3	2	13
19				1	3	4		1 9
20						1	1	1 3
	2	6	11	26	22	24	7	2

V. Left Oral Arm, 4 Days

	9	10	11	12	13	14	15
V. Left Anal Arm, 4 Days							
10					1		1
11							0
12	1					1	2
13		1	1	1			3
14		4	1	1			6
15	1	2	4	5	3	2	17
16		2	2	7	7	2	20
17		1		8	8	8	1 26
18			1	1	7	2	1 12
19				2	3	3	2 10
20						2	1 3
	2	10	9	25	29	20	5

V. Right Oral Arm, 5 Days

	10	11	12	13	14	15	16
V. Right Anal Arm, 5 Days							
10	1						1
11							0
12							0
13		1					1
14			1				1
15	1		6				7
16	2	2	3	2	2		11
17			3	5	2	4	1 15
18			3	9	3	2	17
19			4	2	7	5	18
20			6	2	4	10	3 25
21				1	1		2
22			1				1 2
	4	3	27	21	19	21	5

V. Left Oral Arm, 5 Days

	10	11	12	13	14	15	16
V. Left Anal Arm, 5 Days							
10	1						1
11							0
12							0
13			1				1
14							1
15	1		4	2			7
16	2	2	2	1	3		10
17			3	5	4	4	16
18			3	5	4	1	13
19			1	4	6	6	2 19
20			6	6	9	9	30
21							0
22							1 1
23							1 1
	4	2	21	23	26	20	4

Summary I-V. Left Oral Arm, 2 Days

	6	7	8	9	10	11	12	13	14	15	16	17	18
5		1											1
6							1						1
7													0
8					2		2						4
9						2							2
10			2	4	2	1	4						13
11			1		3	5		1					10
12		1	2	7	5	3	3	4	2	2			29
13	1		4	6	1	3	9	6	2				32
14	2	2	2	6	6	5	12	14	11			2	62
15		1	6	8	10	11	21	13	5			1	76
16	1	2	4	5	13	15	17	8	10	4	1		80
17		2		9	13	19	16	4	5	2	1	1	72
18				3	5	9	17	8	4				46
19				4	4	3	10	9	3	1			34
20				1		4	9	7	8	3			32
21							2		3				5
22								1					1
	4	9	21	53	64	80	123	75	53	12	2	3	1

Summary I-V. Right Oral Arm, 2 Days

	6	7	8	9	10	11	12	13	14	15	16	17	18
6			1				1						2
7						1							1
8			1		1	1	1						4
9													0
10			2	3			1		1				7
11		1	2			4		1					8
12	1	1	1	3	6	5	6	4	3	1			31
13	1		4	4	2	4	10	4	2				31
14	2	2	1	10	4	4	7	16	10			2	58
15		1	6	8	11	11	24	10	4			1	76
16		2	2	5	12	19	18	8	11	4	1		82
17		2	1	12	14	17	14	6	5	2	1	1	75
18			1	1	7	10	17	9	4	1			50
19				7	4	5	13	7	4	1			41
20				1	2	3	7	11	5	2			31
21							1		2				3
	4	9	22	54	63	84	120	76	51	11	2	3	1

Summary I-V. Right Oral Arm, 3 Days

	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Summary I-V. Right Anal Arm, 3 Days																
6							2	1								3
7								1								1
8	1			1		1		3								6
9					1		2		1							4
10	1	1	3			2	2	6	3		1					19
11				5	1	1		1		1	1	2				12
12		1		6	4	3	3	3	3	7	1	1				32
13			1		2	2	1	2	6	6		1				21
14		2	2	15	1	1	7	1	4	10	2	1				46
15			3	10	11	6	8	4	8	6	6			1	1	64
16		1	3	10	13	6	3	10	9	3	4	1				63
17		1		6	14	9	15	6	8	13	4	1				77
18		1	1		6	6	4	3	6	2	1	2				32
19				2	8	5	6	5	5	5		2	1	1		40
20				1		3	1	4	7	7	5	3	5		1	37
21								1	4		4	4	1		1	15
22												5	5	6	1	17
23										1		2	1	1	1	6
24													1		1	3
25													1		1	2
	0	8	11	59	61	45	54	51	64	61	29	25	15	9	7	1

Summary I-V. Left Oral Arm, 3 Days

	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	
6						2		1								3
7						1	1									2
8	1						1	1								3
9				1		4					1					6
10	1	1	4	1	2	3	2	4	1	1						20
11		1	7	2	4		1	3	2							20
12	1		5	7	2	1	3	4	8	1						32
13		2	2	4	1	2	4	3	8	1	1					28
14	2	2	10	4	7	2	4	6	7	2	2					48
15		2	11	12	11	5	5	7	4	6	1	1		1		66
16	1	3	10	12	7	5	7	5	6	5	1					62
17	1		4	13	8	16	4	7	12	3						68
18	1		1	5	4	6	4	5	2		3					31
19				5	3	4	3	6	4	1	2	1	1			30
20			1	1	4	2	4	7	10	2	6	4				41
21						1			2		5	2		1		11
22								1	1		5	5	4	3		19
23									1	1	2	1				5
24											1			1	1	3
25												2				2
	8	11	55	67	53	54	43	60	68	23	30	16	5	6	1	

Summary I-V. Right Oral Arm, 4 Days

	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
5						1	1	1		1							4
6						1	1	1									3
7								2		1							3
8							1		1	1	1						4
9		1			1		1		2	5							10
10				1	2		2	3	1	1	1						11
11				1	2	1	1	5	2	2							13
12	1		3	3	10	10	3	2	7	5	1						45
13				6	5	1	3	6	3	1	2	2					29
14				2	16	13	3	12	5	3	1						55
15				4	18	8	15	19	7	4	3	1	1				80
16					8	6	9	8	10	6					1		48
17		1			3	6	1	15	6	14	8	5					59
18			1				2	2	6	4	7	1					23
19					1	1	1	2	4	4	1	5	3		1		23
20										3	7	9	8	3			30
21												1	4	3	3	1	12
22													8	6	3	3	21
23												1		4	2	6	13
24														1	1	4	7
25																6	6
26																	0
27																	0
28															1		1
	1	2	4	16	65	49	44	78	54	55	32	25	24	17	12	20	2

Summary I-V . Left Oral Arm, 4 Days

	6	5	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	
5						1	1	1		1								4
6						1	1	1										3
7	1							2	1	1								5
8							1	1	1	1								4
9		1			1		1		3	3								9
10			1	1		1	2	4	5	2	1							17
11					4	3	2	5	2	1	1							18
12			2	4	8	11	2	3	3	6	1	1	1		1			43
13				7	9	4	3	5	1	1	4	1						35
14				1	14	12	5	9	4	3	2							50
15				3	17	12	14	19	9	2	5	1						82
16					7	7	5	11	9	4		1			1			45
17		1			2	5	1	14	11	12	5	2						53
18			1				1	3	7	3	7		1					23
19					1	1		4	4	3	3	4	2	1	1			24
20										4	7	6	8	3		1		29
21										4	7	1	3	3	3	2		12
22										1	1		7	5	3	2	1	20
23														3	4	4		11
24														1		6		7
25															1	4		5
26																		0
27														1				1
	1	2	4	16	63	58	39	82	60	48	37	17	22	17	14	19	1	

Summary I-V. Right Oral Arm, 5 Days

	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
<i>Summary I-V. Right Anal Arm, 5 Days</i>																				
9	1	2	1	1			1													6
10	1		4	6	3															14
11	1	3	6	4	2		1	1												18
12	2	4	5	5	6		2	3		1										28
13		1	7	8	2	2	5	2	4	1										32
14		1	2	7	4	2	10	5	5	7										43
15			2	7	3	2	13	8	11	11	1									58
16				1	3	5	7	5	9	4	6									40
17					1	3	11	9	9	7	1	1								42
18					1	3	6	10	6	4										30
19							5	4	9	8	2	2								30
20							7	5	5	12	4	3	2	2						40
21							1	1	1	2	2	2	2		3					14
22							2		1	1	2	5	5	6	2					24
23											1	1	3	3	2	1				11
24											3	2	5	1	2	1				14
25							1				1		2	1	8	6	6	1		26
26													1			2	2			5
27															1	1	10	3	2	2 19
28																	1			2 3
29																		1		1 2
30																		1		1
	5	11	27	41	29	11	72	53	60	58	20	14	18	14	21	11	21	7	2	5

Summary I-V. Left Oral Arm, 5 Days

	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
8	1																			1
9		2	1	1	1		2													7
10	1	1	5	6	2					1										16
11	2	3	5	5	1			2	1		1									20
12	2	5	5	6	5	1	6	3	1	1										35
13			6	6	2	1	5	2	5	1										28
14			2	7	3	2	10	7	5	6										42
15			2	8	5	2	11	7	8	10	1									54
16				1	3	5	4	3	9	7	4	1								37
17				1	1	1	13	8	11	5	1	1								42
18				1	3		6	6	6	3	1									26
19							2	6	8	9	5	1								31
20							7	9	10	11	1	2	4	1						45
21							1			2	1	2	1	2	3					12
22				1					1	2	2	5	4	6	2					23
23											2	2	3	3	2					12
24													3	2	5	2	3	1		16
25								1				1	1	1	12	4	5			25
26												1				1	2	1		5
27															1	2	6	4	2	18
28																	1			1
29																		1		2
30																		1		1
	6	11	26	43	26	12	67	54	65	58	19	16	16	15	25	9	17	8	2	5

STUDIES IN THE DEVELOPMENT OF THE PIPERACEAE¹

I. THE SUPPRESSION AND EXTENSION OF SPOROGENOUS TISSUE IN THE FLOWER OF PIPER BETEL L. VAR. MONOICUM C. DC.

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SEVENTY-ONE FIGURES²

In an account of the seed development of *Heckeria umbellata* (L.) Kunth (*Piper umbellata* C. DC) and of *Piper medium* Jacq., published eight years ago (Johnson, '02), these forms were compared with other angiosperms, and especially with *Peperomia pellucida*. A study of the plant here described was begun at the same time. This species of *Piper* was chosen because, from all species available at my collecting ground, the east end of Jamaica, this one was markedly distinguished by its climbing habit, its unisexual flowers and its immersed ovaries and seeds.

The detailed investigation of the present species confirms certain conclusions reached in the study of the genus *Piper*, but also shows several important peculiarities, especially in the development of stamens and ovaries.

The material used was collected in Jamaica by W. C. Coker in 1900, by the author in 1903 and 1906, by R. K. Miller in 1905 and by I. F. Lewis in 1906. A fifth lot of material was collected in the East Indies by D. H. Campbell in 1907. Obligation is here acknowledged to the above mentioned gentlemen, and also to M. Cassimir de Candolle for identifying the plants from Jamaica,

¹ Investigation prosecuted with the aid of a grant from the Botanical Society of America and one from the Bache Fund.

² Contribution from the Botanical Laboratory of the Johns Hopkins University, No. 15.

on which the results here given are primarily based. The material was fixed in chrom-acetic or in chrom-osmo-acetic.

The flowers of *Piper betel* are formed in long terminal spikes. The individual spike may be made up partly or wholly of pistillate flowers with occasional staminodia, wholly of staminate flowers, or, more rarely, almost wholly of hermaphrodite flowers (fig. 13).

The mature staminate flowers are about two millimeters in diameter and five centimeters or more in length. The female and hermaphrodite spikes are often twelve millimeters thick when mature, and as long as the males. The number of flowers on a spike may be as great as 500 or 600. On either a male, female or hermaphrodite spike there may be from four to ten sterile bracts at the base. Above these, the lowermost fertile bracts, on the hermaphrodite spike, may bear hermaphrodite flowers just as often as male or female ones. The conditions at the tip of the spike are apparently similar, but the number of sterile bracts is more variable. Sometimes all but two or three of the terminal bracts have flowers in their axils, while, in other cases, twenty or more of the terminal bracts may be sterile.

Each hermaphrodite flower consists of two stamens, which stand side by side, at the same level on the spike. The carpels, with the three or four stigmatic lobes, stand between them (figs. 4, 5, 13, 44). Each flower is subtended by a bract, which is at first rather bracket-like with a short thin stalk (fig. 1). Later it becomes somewhat mushroom-shaped, with a thick stalk and a nearly circular terminal scale (figs. 34, 50).

The development of the flower is initiated in the usual way, by the bulging out of the perilem to form the stamen. Each stamen soon shows a swollen sporogenous tip, flattened on the side toward its mate, and a short but distinct stalk below (figs. 3, 4). The archesporium of the stamen arises as usual from groups of perilem cells, and is already well differentiated when the carpellary ring is closing above the ovarian cavity (figs. 2, 4).

When first clearly distinguishable, the primary archesporium of each microsporangium consists of a rounded group of 50 or more cells (figs. 8, 9). These divide later to form 1000 or more

spore-mother-cells in the larger microsporangia (fig. 11). The development of the tapetum and tetrads of microspores shows no unusual features. The ripe microspores are spherical, 7-9 μ in diameter, with a smooth outer wall. They are at this time unicellular, but have two nuclei. When the nuclei are first formed, a delicate evanescent wall is visible between them. One of the cells thus formed is about twice as large as the other (figs. 18, 19).

The wall of the anther is three cells in thickness, except at the top, where it may be but two, outside the tapetum. The opening of the anther for the escape of the pollen is a slit or gap, which is terminal to the style, and is situated at the base of the spike (fig. 45). With the ripening of the anther the style of the stamen elongates considerably, and the anthers are thrust out beyond the bracts. The bracts, however, have been elongated markedly by the increase in thickness of the axis (fig. 46).

The most interesting feature of the development of *Piper betel* is the variability in the development of the stamen as a whole, and of the microsporangia in particular. This occurs in *Satureia*, as is illustrated by C. S. Gentry (fig. 32). Often one of the two stamens of a male or hermaphrodite flower in *P. betel*, is much smaller than the other. In some cases, in a surface view, or section, one of the stamens is not infrequently one of the stamens of an hermaphrodite flower is reduced to a sterile knob, or merely a trace of a microsporangium (figs. 32, 34). In some flowers, of both female and hermaphrodite spikes, both stamens are reduced to this condition (fig. 32).

In the hermaphrodite flowers of *Piper betel*, the appearance of the stamens and ovules in section shows that both are functional in the same flower. If this is the case, the plants should be considered polygamous, rather than monogamous.

If we now consider the stamens which do contain microsporangia, we find that the size and extent of the microsporangia differ greatly in different flowers, or even in the same flower. A count of the sporangia in the stamens, from five hermaphrodite flowers and from five male flowers, showed only 13½

per cent of these stamens had the usual complement of four sporgangia (figs. 10, 16, 20). About 22 per cent of them had three sporogenous masses each (figs. 21, 22, 31). The larger proportion, 37½ per cent of all the stamens, had only two groups of sporogenous cells, which may both be in the same theca (figs. 26, 27, 28), or on opposite sides of the connective (figs. 23, 24, 30). In a very considerable proportion of the stamens but a single pollen mass is formed. This was found in 17½ per cent of those counted. The sole remaining spore mass may be on either the acroscopic or the basiscopic side of the connective (figs. 27, 28), or it may extend past the connective from one theca to the other (figs. 25, 29, 31). In all cases of this sort seen, the mass was continuous across the ventral side of the stamen (figs. 29, 31). Finally, 10 per cent of the stamens counted were devoid of any sporogenous tissue whatever (figs. 32, 33, 34, 35).

Not only does the arrangement of the sporogenous tissue differ in the stamens, but serial sections show that it differs at different points in the same anther (figs. 22, 23—fig. 23 being nearer the anther).

A reduction in the number of spore masses can be distinguished among the stamens counted in figs. 20 to 32. There is a small number of cases in which the masses present is in the position of the four sporangia of the normal stamen, while one or more of the regions usually occupied by a sporangium remains empty (figs. 9, 26). In the second series of cases the anther is usually occupied by only two or three distinct sporangia, together with the larger or smaller reduction between these, is occupied by a continuous sporogenous tissue (figs. 21, 23-25, 27-29). In the stamens were seen in which the continuous sporogenous tissue seemed to fill the regions of the anther by all four of the sporangia of the normal stamen.

The first reduction mentioned is the one which can be taken as an example of the phenomenon referred to by Goebel (1902, p. 200) as the "arrest or suppression of pollen sacs," and also mentioned by Bower ('08, p. 127). A portion of the stamens in which Cases of this

be used, in the case of *Piper betel*, at least, to indicate any ontogenetic process of union occurring after the sporogenous cells are once distinguishable.

If the normal stamen of angiosperms is to be regarded as possessing four microsporangia—a view generally accepted—then it is evident that in the case of certain of these abnormal stamens we are warranted in saying that the development of one or more of the microsporangia is completely suppressed. The tissue, which in the normal stamen organizes the sporogenous mass, may in another fail to show any such specialization of its cells. On the other hand, we have seen that the tissue of the region which usually forms the connective may occasionally give rise to sporogenous cells, and thus form a continuous pollen mass across from theca to theca (figs. 25, 29, 31).

In the above description I have spoken generally of sporogenous masses, instead of sporangia. The question naturally arises here whether each distinct sporogenous group, with its continuous tapetum and wall, is to be regarded as an individual sporangium. In stamens, such as are illustrated in figs. 9 and 26, one must admit it would seem, that two microsporangia are present, and that each corresponds exactly, in location and development, to one of the four sporangia of the ordinary stamen. When however, we attempt to determine the number of sporangia in stamens such as are shown in figs. 21, 24, 25, 27, 28 and 29, the solution of the problem is not so evident. If we accept the definition of sporangium given by Bower ('08, p. 112), that an individual sporangium, in vascular plants, consists essentially of "an isolated spore-mother-cell, or a connected group of them, or their products—together with its protective tissues," then in the stamen shown in fig. 27, for example, there is but a single microsporangium present. For there is a single continuous mass of sporogenous cells completely surrounded by a tapetum. If, on the other hand, we note the position and extent of the sporogenous mass, and its partial division by a septum, it then seems evident that it is in position and extent the equivalent of two of the sporangia of an ordinary stamen. For the same reasons, the sporogenous mass of the stamen shown in fig. 29 may be considered the equivalent

topographically, if three microsporangia, though according to Bower's definition, it would be a single sporangium.

The outcome of his consideration of the facts stated must be to emphasize the lack of any definiteness in our conception of the sporangium as an individual organ, a lack which has been most clearly indicated, perhaps, in recent literature, by Bower's definition, above quoted.

The suppression or reduction in development of microsporangia may, as we have seen, be carried so far that the stamen is left quite sterile (figs. 32, 34). In still other cases, as in some female flowers, the stamen is apparently not represented by any structure, not even a staminodium. Even where pollen is formed it appears sometimes not to be functional, if one may judge from the withered microspores seen in flowers where other tissues seem well fixed.

The question of the greatest importance in connection with the present study is that concerning the factors which change the course of development of certain stamens in such a way that the normally fertile portions remain sterile, or, in other cases, the cells of normally sterile regions become fertile. This question will be considered after we have noted the development of carpels and ovules.

The wall of the ovary in *Piper betel* arises as a ring-like but lobed outgrowth, at a point just above the subtending bract of the flower. It is therefore between the two stamens, in the case of the hermaphrodite flower (figs. 2, 4, 13, 33, 44). Soon after this circular wall has closed in above the ovarian cavity, the ovule appears as a slight mound at the bottom of this cavity (fig. 2). The lobes of the ovarian wall elongate considerably as growth proceeds, and give rise in the mature flower to the three- or four-lobed stigma (figs. 13, 25, 35, 36, 37). The lobes of the ripe stigma are somewhat pointed and papillate (fig. 44), but after pollination, they thicken at the base and shrivel at the point, till in the mature fruit nothing but three or four blunt warty tubercles remain (figs. 65, 66). The number of these lobes usually present at the tip of the carpellary wall, and the presence of three partial divisions in this wall, some distance below the tip, indicates

that this wall is composed of three carpels (figs. 36). This view is strengthened by the fact, that even when a larger number of stigmatic lobes is present, the structure of the wall lower down is like that of ovaries with three stigmas. For example, a transverse section nearer the base of the ovary from which the four stigmatic lobes in fig. 37 are taken, shows a structure exactly like that illustrated in figs. 35 and 36, which latter figures are taken from an ovary with a distinctly three-lobed stigma. The number of vascular bundles in the wall of the ovary also indicates that it is made up of three carpels. All of this evidence supports the interpretation of the structure of the ovary given for the close relatives of *Piper betel* by Eichler ('78, 2, fig. 4).

From a very young stage of development of the flower, the tissues of the axis continue to expand radially on all sides, keeping pace with the elongation of the ovary. The result of this is that the cavity of the ovary becomes completely buried in the fleshy axis (figs. 4, 33, 44, 63, 68). Another result of this growth is that the stamens are carried outward, and often in the mature flower, seem to stand upon a mound of carpellary tissue (figs. 44, 45, 65).

The structure of the ripe fruit of *Piper betel* differs markedly from that of *Piper medium* (Johnson, '02, p. 322, figs. 14, 15). In the first place, the immersion of the seed of *P. betel* in the axis leaves little of the tissue about it to be derived from the carpels proper. The latter apparently form only the roof of the ovarian cavity (figs. 65, 66). The three layers of the carpellary tissue of the fruit of *P. medium* are distinguishable in *P. betel* only at the upper end of the fruit. At the sides, and near the base, only the two inner layers are present. There are four vascular bundles running longitudinally in the outer of these two layers (fig. 67), instead of six as in *P. medium*. The oil-secreting cells are three or four layers thick in the upper third of the fruit (fig. 66), they form a single broken layer at the sides of the fruit, and become more numerous again at the base. The whole of the tissue surrounding the seed is relatively soft, and the seed apparently must be set free by the decay of these.

While the normal course of development of the fruit in a female or hermaphrodite flower, is that just described, there are scattered

over the male, female and hermaphrodite spikes, female flowers, which show a wide range of stages of degeneration (figs. 46-51). Moreover, in any flowers with normal ovaries, the ovules seem to degenerate after reaching various stages of development. This often begins before the time of pollination and hence cannot in these cases, at least, be attributed to the lack of a stimulus due to this process. The first stage noted in the series showing progressive degeneration of the ovary itself is one in which, though the carpels have closed together above the ovarian cavity, and the stigmas seem normally developed, the ovary is without a trace of an ovule (fig. 46). In other cases the ovarian cavity may be as reduced as in those just mentioned, and in addition, the stigmatic lobes markedly retarded in growth (fig. 47). In the flower shown in figs. 48 and 51 the ovarian cavity has entirely disappeared, while the stigmas are represented by a small spine, with no trace of three distinct constituent lobes. The stigmas of the flower shown in fig. 49 are represented merely by a slight mound. Finally, in the flower shown in fig. 50, there is no trace whatever of carpellary tissues.

The above mentioned cases were taken from spikes in which most of the flowers seemed functionally hermaphrodite, and in all the flowers figured the pollen seemed to be perfectly normal.

Of the functionally male spikes studied, only two or three spikes were found in which the ovary was not represented by a slightly or considerably developed rudiment, in from 5 per cent to 30 per cent of the flowers. Often an ovarian cavity is present, and rarely a well-organized ovule. No case, was seen, however, where fertilization seemed to have occurred in an ovule on a male spike.

The ovule of *P. betel* arises as a mound on the floor of the ovarian cavity. It is first distinguishable just after the carpels have closed in to surround the cavity (figs. 2, 4).

The inner integument is initiated at about the time the primary sporogenous cell is undergoing its first division (figs. 33, 52). The tips on the carpels have at this time closed tightly above the ovule to form a rather long stylar canal (figs. 45, 52). The outer integument starts soon after the inner (fig. 53), but never grows above the latter to take part in the formation of the micropyle,

as it does in *P. medium* (Johnson '02, figs. 5, 11, 14). A striking feature in the rate of growth of the integuments in *P. betel* is the inequality in rate of growth on the different sides of the ovule. That this difference is very considerable is evident from many longitudinal sections of the ovule (figs. 44, 54). The minor irregularities are still more clearly shown by the study of a series of successive transverse sections, such as are shown in figs. 38 to 43. This series shows that the inner integument has two distinct lobes, while the outer one has at least four. The longest lobe of the outer integument is pushed up tightly against the lower end of the stylar canal (figs. 44, 54).

In the ripe seed, the outer integument is made up of four or five layers of thin-walled cells (fig. 61). The inner integument forms the chief seed coat. It consists of three or four layers of cells throughout most of its extent. The innermost of these is slightly thickened and brownish in color, while the outermost layer has its outer walls greatly thickened by a granular deposit against their inner surface (fig. 68).

The escape of the seed from the fleshy spike, and the germination have not been seen. It is hoped that these will be found in material to be collected this spring.

The embryo-sac arises from the single, hypodermal, sporogenous cell in each ovule. This cell cuts off a thin parietal cell at the micropylar end, which may form six or seven layers of tapetal cells at the tip of the mature nucellus (figs. 52, 54, 57). The lower of the two descendants of the primary sporogenous cell enlarges, and finally develops directly into the embryo-sac (fig. 54). The first mitosis in the sac has not been seen, but when the two daughter nuclei prepare for division (fig. 55), it is evident that only sixteen, the reduced number of chromosomes, is present.

The four nuclei resulting from this second division of the embryo-sac may sometimes remain near the ends of the sac, where they are formed, or there may be a single nucleus at one end and three at the other (figs. 56, 58). Often, however, perhaps in half the cases seen, these nuclei may be closely grouped near the middle of the embryo-sac (fig. 57). In these cases the nuclei are as closely grouped as the nuclei of a microspore tetrad. It is of

course possible that some of these variations from the usual arrangement of nuclei in the four-nucleate stage, are connected with the phenomenon of degeneration of the sac which is of not infrequent occurrence, to judge from shrunken sacs found in the older spikes.

In an early eight-nucleate stage the nuclei are found gathered in two groups of four each, one at the upper, one at the lower end of the sac (fig. 59). Somewhat later than this an egg apparatus, of the usual three-celled type, is found at the upper end of the sac. Three antipodals are grouped at the base, and two polar nuclei meet at the middle of the sac (fig. 60).

Pollen tubes, and the details of fertilization, have not been seen, but, from the number of cases where two nuclei were seen in the egg, (fig. 54), there seems no reason to doubt that fertilization is accomplished in the normal manner. No evidence of a triple fusion has been noticed. The early stages of development of the embryo and endosperm have not been found, but in seeds that have grown to twelve times the diameter of one containing a just-ripe sac, the fertilized egg is still undivided, though the endosperm nucleus has given rise to scores of free nuclei in the peripheral cytoplasmic layer of the sac (fig. 63). The antipodals at this time have already multiplied to a number which may be as great as 35 in a single section of the sac. They occupy a large space at the base of the sac (figs. 61, 62). In the ripe seed the antipodals, somewhat crushed, can still be seen in a depression below the endosperm (figs. 65, 70).

The endosperm develops cell-walls after about 100 or more free nuclei have been formed, the walls apparently arising in the ordinary way. In the mature seed the endosperm forms an irregularly globular mass about 700μ in diameter, and showing about 150 cells in a median longitudinal section of the seed and embryo sac. The cells of the ripe endosperm contain little, if any, starch but have rather dense protoplasmic contents, which may serve as a store of nitrogenous material. The chief carbohydrate supply of the seed is stored in the starchy perisperm, which makes up $99\frac{1}{2}\%$ of the bulk of the seed (fig. 65).

DISCUSSION

The most interesting questions raised by the foregoing work on *Piper betel* are those concerning the different degrees of suppression of sporangia and sporophylls.

From the progressive series of reductions above noted, it seems fair to assume that whatever influence is at work to suppress sporangial development, is also concerned with the complete suppression of the stamens or carpels, and thus determines the formation of male or female flowers, which in many cases, make up the whole spike, to the exclusion of hermaphrodite ones. In other words, it is this influence that determines whether gametophytes of one or both sexes shall be formed at the next step in the life cycle of this plant, and of which sex they shall be.

We can take up these questions in regard to *Piper betel* more profitably after reviewing what is known of other plants, first concerning the time, and second, concerning the immediate cause of the differentiation of sexes in gametophyte or sporophyte.

Of well-known forms among the algae it is clear that in *Vaucheria*, *Nemalion* and some species of *Spirogyra*, *Ædogonium*, *Coleochaete* and *Fucus*, the individual plants are distinctly hermaphrodite, since the two kinds of sexual cells may arise close together on the same plant. In other species of *Spirogyra*, *Ædogonium*, *Coleochaete* and *Fucus* and in many *Chlorophyceæ*, *Phaeophyceæ* and *Florideæ*, the sexual plants bear one kind of sexual organs only.

In such a form as a dioecious species of *Ædogonium*, *e.g.*, the hermaphrodite condition can be supposed to exist, if at all, only up to the formation of the zoöspores from the zygote. It is not known whether all zoöspores from one zygote give rise to plants of the same sex or not. In dioecious species of *Fucus* there is no evidence to show that the thallus is not unisexual from the time of its origin from the oöspore. Hence the hermaphrodite condition must be supposed to exist in the oöspore only, and for a very short time. In hermaphrodite species of *Fucus* we must conceive of the thallus as being hermaphrodite up to the time of differentiation of antheridia and oögonia. If Yamanouchi's ('09)

view is correct, that the so-called antheridia and oögonia of *Fucus* are really sporangia, and that the male and female cells are really the daughter cells of spores that germinate directly in the sporangium, then the segregation of the sexes here, at the initiation of the microsporangia and megasporangia, is very like that to be noted later in the heterosporous ferns.

The case of *Nemalion* differs from that of the monoecious species of *Fucus* in the intercalation of a process of fragmentation of the oöspore (carpospore-formation), during which process, however, no segregation of the sexes occurs. This is evident from the fact that each carpospore gives a plant bearing antheridia and carpogonia on neighboring branches. This fact is particularly interesting in view of the statement of Wolfe ('04), that meiosis does occur during carpospore-formation.

In the brown alga *Dictyota dichotoma* the oöspore gives rise to a hermaphrodite plant which bears tetraspores (Williams, '03 and Hoyt, '10.) The latter germinate to male and female plants, perhaps two of each from each tetrad, according to Hoyt. The segregation of sexes apparently occurs in this species at meiosis.

In most of the Floridæ the conditions existing are probably the same as in *Dictyota*, as is indicated by the work of Yamaguchi ('06) and Lewis ('09). That is, the gametophytes are dioecious, the sporophyte is hermaphrodite, and segregation occurs along with meiosis at tetra-spore-formation.

In the fungi, little work has been done on this problem, except the very interesting work of Blakeslee on the moulds. In the genus *Sporodinia*, Blakeslee ('06) has discovered that the mycelium from both sporangiospores and zygospores is hermaphrodite, and thus that the sexual substances fusing in the zygote must become distinct, if at all, at about the time of the formation of the gametes. In *Phycomyces nitens*, he found that the mycelium from the zygote is hermaphrodite, while that from the sporangiospore is generally unisexual, and the segregation of sexes occurs during the development of the spores. Finally, the same worker found that in *Mucor mucedo* the mycelium from each zygote, like that from the sporangiospores, is preponderatingly male or female,

and the segregation of sexes, or suppression of one sex must therefore occur between the fusion of gametes and the germination of the zygospore, and must result in the suppression now of the male, now of the female tendency, in the mycelium coming from the zygospore.

Among the Bryophytes, it seems clear from the work of Blakeslee on *Marchantia* ('06), the Marchals on mosses ('06, '07), and of Strasburger ('09) on *Sphaerocarpus*, that the segregation of sexes takes place at spore-formation, probably during meiosis.

In homosporous ferns each sporophyte is usually clearly hermaphrodite, each spore also, and the prothallus coming from it, is usually bisexual, for though the male organs only may be developed at first, the female organs are usually formed later, on all well-nourished prothalli. Segregation in this case, if it can be called such, evidently takes place during the later development of the gametophyte.

The heterosporous ferns and *Selaginella* give the first clear indication, after that noted in *Mucor mucedo* and possibly *Fucus*, of the segregation of the sexes at a point in the development of the sporophyte other than sporogenesis. In *Marsilia*, *e.g.*, the gametophyte is never hermaphrodite, but distinctly male or female. The sporophyte, on the contrary, is hermaphrodite and retains this condition, so far as can be seen, up to the time of the separation of the three marginal cells of the seventh grade, in the sporocarp, which give rise to all the thirty or forty microsporangia and megasporangia of each sporus (Johnson, '98, figs. 34, 38). Shattuck ('10, p. 23) states that all the sporangia of *Marsilia* have the same development up to the time that spore-mother-cells are formed, after which the microspores and megaspores are markedly different in character. He states further that changing the conditions surrounding the plant, at the proper time of development, may cause the young spores in certain of the microsporangia to become enlarged, and to assume somewhat the type of spore wall of the normal megaspore. He has not, however, yet been able to germinate these enlarged spores, and has therefore failed to demonstrate conclusively that these have

become really female in character. It seems to the writer, especially in view of the early distinction of the micro- and megasporangium initials in *Marsilia* (Johnson '98), that the view of Strasburger ('09, p. 12), is still tenable—that all the spore-mother-cells formed in the microsporangium are essentially male and those in the megasporangium are essentially female.

Seliganella resembles *Marsilia* in that the gametophytes are unisexual and the sporophyte hermaphrodite, but it differs in that the sexes become distinct considerably earlier in the development of the sporophyte than in *Marsilia*. In fact, the sporophylls bear one kind of sporangium each. In erect spikes, those sporophylls at the base of the spike bear megasporangia only, those at the tip, microsporangia only. In the prostrate spikes of other species, megasporangia occur on the sporophylls turned toward the earth, while in species with drooping spikes they are found at the tip only (Hieronymus ('00), p. 659).

In such conifers as *Pinus* and *Larix*, and in monoecious angiosperms, the gametophytes are male and female, and the sporophyte is hermaphrodite, but the separation of the sexes is evident at a still earlier stage than in *Selaginella*. Microsporangia and megasporangia are borne not merely on different sporophylls but even on different branches, that is, in male and female cones or flowers.

In *Cycas* and *Ginkgo* and in dioecious angiosperms, the segregation of the sexes occurs at a still earlier stage, and male and female flowers are developed on entirely separated sporophytes, that is, the sporophyte, as well as the gametophyte, has become apparently unisexual, like the zygosporic mycelium of *Mucor mucedo*. In these angiosperms the hermaphrodite condition exists, if at all, only in the fertilized egg, or, possibly, on into the early stages of the sporophyte. It is certainly evident that the sex of the sporophyte is fixed, once for all, at some stage of the sporophyte before that at which the first crop of flowers is borne. For, so far as the writer has been able to learn, perennial plants of this type bear flowers of the same sex year after year.

In most angiosperms, while the gametophytes are unisexual, the sporophyte is hermaphrodite, so far as can be seen, up to the

time of formation of the sporophylls, when a separation of the sexes is evident. Here, as in the case of the gametophyte of the fern, the male influence or substance, seems dominant at first, as is evidenced by the earlier origin of the microsporophylls in most instances. These angiosperms differ in this respect from those Selaginellas with basal, (i.e. older), megasporophylls and terminal microsporophylls, though they resemble these selaginellas in having both megasporangia and microsporangia on the same axis. The monoecious Araceae, among angiosperms, also offer an example, or, at least, evidence, of the earlier dominance, of femaleness, in the usually basal position of the female flowers, on the spadix. Cases have also been noticed by Correns ('08), in *Satureia*, and by Strasburger ('09²) in *Mercurialis*, where purely female flowers are the first to develop in the inflorescences of these plants, the stamens not developing until much later.

We now come to the second part of our question, namely, the cause of the segregation of the sexes at the particular point where it does occur.

Experimental work on a number of plants has shown pretty clearly that the distribution of nutrient substances in the plant, together with the external conditions affecting the nutrition of the plant as a whole, are among the important factors concerned in the expression or suppression of certain organ-building tendencies in plants. We may recall in this connection only such of this work as bears on the causes of development of the reproductive organs.

Klebs has shown that the development or non-development of the sexual reproductive organs of *Vaucheria*, some other algae, and certain seed plants can be determined by changing such external factors as the osmotic or chemical character of the nutrient solution, or of the light or temperature affecting the plant. These facts strongly suggest that such factors may also determine which sex may be assumed by any individual in dioecious plants.

Among higher plants, such as ferns and *Equisetum*, many observers have asserted that the smaller, poorly nourished gametophytes are always male. In view of the fact that the antheridia appear on normal prothallia before the archegonia, the persistent

absence of the latter organs in certain cases may simply mean that these starved prothallia fail to attain that degree of maturity (whatever that may mean) at which archegonia are normally produced. This view seems distinctly confirmed by the experimental work of Miss Wuist ('10), on the prothallia of *Onoclea struthiopteris*. Campbell ('05, p. 314) has stated that these prothallia are constantly dioecious. Miss Wuist finds that ordinary soil cultures show about 1 per cent of protogynously monoecious prothallia. When prothallia, from a soil culture, which have borne only archegonia, are grown in Beyerinck's fluid for five to seven days they begin to bear antheridia. Similar results were obtained when prothallia bearing archegonia only were transferred from distilled water to Knop's solution. Miss Twiss ('10, p. 168) has shown that the prothallia of *Aneimia* and *Lygodium* are not really dioecious but merely protandrously monoecious. Goebel ('05, p. 220) suggests that the evidence for persistent dioeciousness in the ferns is everywhere inadequate.

It seems possible, from what has been said, that all the cases of dioeciousness described among homosporous pteridophyta are attributable to external factors. This belief is strengthened by the work of Correns ('08, p. 661) on *Satureia hortensis*. He has shown that, in this gynomonoeious species, individuals that under normal conditions produce 15 per cent of normal females, 7 per cent of imperfectly hermaphrodite and 78 per cent of functionally hermaphrodite individuals can, by cultivation on poor soil, or with insufficient illumination, be induced to form a larger percentage of pure females. By the combined action of these two agencies the male tendency may be so greatly inhibited that but 17 per cent of hermaphrodite flowers are formed while the proportion of pure females rises to 79 per cent. Correns also notes that the percentage of hermaphrodite flowers is greatest at the height of the blooming season, while, at the beginning and end of the flowering period, female flowers preponderate.

Another interesting case with a bearing on this question, and one that perhaps brings us a step nearer the immediate causal factor, is that of *Mercurialis annua*, studied by Strasburger ('09¹, p. 507). He found that certain isolated plants of this species

persisted for months in bearing female flowers only. Finally a few reduced stamens appeared, and then, after the process was once initiated, the same plants continued to bear considerable numbers of short-lived, but functional, stamens along with the female flowers. Certain of the plants, bearing female flowers only, had these accidentally pollinated from a male plant in another greenhouse. In consequence, they not only formed seeds, but, shortly after, began to develop male flowers, and did this much earlier than did the unpollinated female plants mentioned above. These facts led Strasburger to the conclusion that the lack of pollination, and the consequent lack of seed-development in this species, leads to a gradual increase of a tendency to initiate stamen rudiments, probably by the accumulation of some material substance. The same process may be induced even more quickly by the influence on the female plant of pollination and seed-production.

The works of Williams ('03) and Hoyt ('07) on *Dictyota* suggest a similar accumulation of some activating substance as the cause of the very regular periodic initiation and discharge of the gametangia of this alga.

In *Melandryum rubrum*, Strasburger ('00) found that the presence of the parasite, *Ustilago violaceae*, may cause plants that have hitherto borne only female flowers to develop staminate ones. Goebel ('07), suggests that, in such cases as this of *Melandryum*, the course of nutritive substances in the inflorescence is changed, in consequence of some stimulus produced by the *Ustilago*. Strasburger ('09, p. 19) suggests that a substance activating male development, and one activating the development of female organs, are always present in the inflorescence, and that in hermaphrodite flowers the two substances come into play separately, while in male or female flowers the male substance alone, or the female substance alone, completely preponderates.

Whether the explanations suggested by these unusual types can be assumed to indicate the relation of the sex-determining substances in the case of permanently unisexual plants is, however, less certain. This is shown, *e.g.*, by the work of Noll on *Marchantia*, of which he propagated the unisexual plants by the gemma

for over thirty generations without any indication of change of the strictly unisexual condition. It must also be remembered that such perennial dioecious plants as *Cycas*, *Ginkgo*, *Populus*, etc., have been under continuous observation for years, without any completely authentic case of change of sex being recorded, so far as the writer has been able to learn. It may be considered as doubtful whether the apparent microspores found by Chamberlain ('97) on the carpel of *Salix petiolaris*, the apparent spermatogenous cells found in the archegonia of *Mnium* by Holferty ('04), or the apparent megaspores found in the microsporangium of *Marsilia* by Shattuck ('10) are really capable of functioning as reproductive cells.

We may now consider the case of *Piper betel* in the light of the observations just reviewed, to see whether these help in interpreting the facts recorded concerning this species, and also whether these facts support or controvert in any degree the views reached from the study of other forms.

It is clear, from what has been said of *Piper betel*, that the sporophyte of this species is distinctly hermaphrodite, and also that the sexual character of each constituent of the hermaphrodite flower is already determined at the time of initiation of the stamens and carpels. It is likewise evident that the tissue of the young spike that is to bear perfect flowers must be potentially hermaphrodite in character. If this latter be true, then it seems probable that the tissue of those spikes, all of whose flowers are functionally unisexual, is likewise hermaphrodite at first. We must either admit this as proven by the fact that staminate flowers often bear some rudiments of megasporophylls, and the carpellate flowers nearly always bear rudimentary stamens, or else we must assume that, in the latter case, *e.g.*, the male-determining substance is absent but the female-determining substance is capable of causing, or at least allowing, the development of the abortive stamen-like structures. Of these two possibilities, the evidence available seems to make the former view far more probable. In other words, it seems clear from the different degrees of suppression of microspogenous tissue described in *P. betel*, that we must assume the male-determining substance to be present in all flowers, but more

or less inhibited in certain flowers from expressing itself as a controlling factor in the development of tissues in the flower rudiment. That is femaleness is unusually more or less dominant over the male tendency. (Shull, '10, p. 119.) Or, we must assume that an absolute segregation of the sex-determining substances may occur at very different times in different spikes, in different flowers of the same spike, or even in different stamens of the same flower. Of these two views the former seems much more probable from the facts above given. It also agrees with the results, previously referred to, of the work of Correns ('08) and Strasburger ('09) on gynomonoeious angiosperms. The same condition is clearly indicated also by the work of the Marchals ('07) on those diploid gametophores of the mosses, in which, while some individuals bore both antheridia and archegonia, *i.e.*, the flowers were hermaphrodite, other individual gametophores, as long as kept, bore only one kind of sexual organ each.

From all the evidence now available we are warranted in assuming the possibility of the presence of the second sex in many of those angiospermous sporophytes where only one sex has thus far been detected. The best evidence for this assumption being found in the fact that in certain known cases the application of the proper stimulus may cause this second sex to become evident, by the development of its proper reproductive organs. (Morgan, '09, p. 337, 346.)

If then the flower of *Piper betel* is potentially hermaphrodite, what is the cause of the suppression, partially or wholly, of the sporogenous tissue or even of the sporophyll itself, now of the stamens, now of the carpels or now of both? What, in certain stamens, is the cause of the extension of the sporogenous tissue across the whole width of the anther? That space relations, or the crowding of the parts of the flower in the bud, do not constitute the determining factor seems evident from the fact that the sporogenous tissue of the microsporangium, *e.g.*, may be suppressed or extended now in the upper, now in the lower theca of the stamen, while, less frequently, it may occur in both, or extend across from one theca into the other (figs. 9, 24-31). There is likewise no indication of the localization of these abnormal

flowers along the axis of the spike. They occur with equal frequency at the base, middle or tip of the spike. Finally, it is possible that the immediate cause of the failure of a microsporangium, *e.g.*, to develop in any quarter of the anther is the absence of the necessary nutritive or spore-determining material from this region. How such an unusual distribution of this substance may be brought about it is not easy to see, though it is perhaps no less understandable than the cause of the usual distribution which results in the formation of the four microsporangia in the normal stamen.

Probably any factor that disturbs the mechanism for the normal process sufficiently may bring about the suppression or extension of sporogenous cells. Such a disturbance of the normal movement of either nutritive, stimulative, or possibly of inheritance-bearing substances in the plant seems the most probable immediate cause of the phenomena here recorded for *Piper betel*. Moreover it seems evident that the change which occurs in this sex-determining or stamen-determining substance is progressive and quantitative. (See Morgan, '09, p. 336.) Whether this distribution of material is ultimately conditioned by external factors acting on the plant, must here, as elsewhere, be determined by experiment. For an investigator working in a region where this plant can readily be brought to flower and fruit, it may be expected to yield results of great interest concerning the segregation of the sexes in this species, with a bearing on the problem of the distribution of sexes in angiosperms generally.

SUMMARY AND CONCLUSIONS

The distribution of flowers of *Piper betel* may be either dioecious, monoecious, or monoeciously polygamous. The degree of development of the stamens and pistils often differs markedly in different flowers of the same spike, or even in different stamens of the same flower.

The details of the development of the stamens and pistils of the perfect flowers are those usually found in angiosperms. An evanescent wall separates the two nuclei formed by the first

division of the microspore nucleus. The ripe microspore is unicellular and binucleate. The primary archesporial cell of the ovule cuts off a single parietal or tapetal cell above. Then the lower half, which is perhaps to be considered a megaspore mother-cell, gives rise immediately to an eight-nucleate embryo-sac of the usual angiospermous type.

Fertilization and endosperm-formation take place in the normal way. About 100 free, peripheral nuclei are formed before cell walls appear in the endosperm. The antipodals multiply to 100 or more. In the ripe seed the embryo consists of a globular undifferentiated mass of about 500 cells. The endosperm is of 150 cells in median longitudinal section. Its cells contain little stored carbohydrate. The numerous antipodals persist in the seed, but seem to have little stored food-material in them. The abundant and starchy perisperm is the chief storage tissue. The fruit is immersed in the axis, but aside from the differences in structure connected with this fact, it resembles the fruit of *Piper medium*.

The most striking peculiarity of this species is the extreme variability in the development of the microsporangia and megasporangia on different spikes or in different flowers of the same spike. The number of microsporangia in a stamen *e.g.*, may vary from none to four, and the extent of a single sporangium may be such as to fill one-quarter of the anther, or, in others, as much as three-quarters. The number and relative extent of the sporogenous masses in the stamen is constant from the time of their initiation. There is no breaking down later of sterile septa to throw two sporogenous masses into one. There is no secondary fusion or confluence of sporangia, nor is there any evidence of the abortion or suppression of sporogenous tissues once initiated, unless it be found in the possible infertility of the well-formed pollen of some of the ripe anthers.

The cause of the differences in development of the sporogenous tissue seems not to be connected in any way with the space relations of the flowers on the spike, since any type may occur at any point on the spike, *i.e.*, at base, middle or tip. The real cause is probably to be sought in those factors, internal or external,

that disturb the normal production or course of movement of material in the plant.

The evidence from this study of *Piper betel* concerning the distinction of sexes in the sporophyte, seems to show that the tissue of the young spike, and often of the individual flower, must be hermaphrodite in character. The differentiation of the sexes, by separation or by the suppression of one of them, must take place at or after the initiation of the rudiments of the parts of the flower. This view is in accord with the conclusion gained from experimental work on a number of species of angiosperms, as well as on certain lower plants.

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EXPLANATION OF FIGURES

All figures are camera drawings and all are from microtome sections except figs. 13, 25, 65, 68.

The magnification given in the description of each figure is that actually shown by the figure as printed on the page.

Abbreviations used: *Ant*, antipodal; *Asp*, archesporial cell or cells; *Br*, subtending bract; *Cp*, carpel or carpellary tissue; *E*, egg; *Em*, embryo; *Esp*, endosperm; *In*, inner integument; *Oc*, oil-containing cell; *Oin*, outer integument; *Pa*, parietal cells; *Ps*, pollen-sac; *Psp*, perisperm; *St*, stamen; *Vb*, vascular bundle.

1 Part of longitudinal section of young spike, showing bracts and rudiments of stamens. $\times 75$.

2 Part of similar section of slightly older spike, showing rudiment of carpels and ovule. $\times 75$.

3 Part of section like last, through a stamen showing beginning of differentiation of the archesporium. $\times 350$.

4 Longitudinal section of flower through stamens and carpels. $\times 110$.

5 Transverse section of a bract and the two stamens of its flower. $\times 160$.

6 Transverse section of very young stamen. $\times 600$.

7 Transverse section of slightly older stamen, showing beginning of differentiation of archesporial and vascular tissue. $\times 350$.

8 Similar section of slightly older stamen, showing one archesporial group of cells at each end of the section. $\times 350$.

9 Similar section of still older stamen, showing two archesporial masses in one loculus and none in the other. $\times 350$.

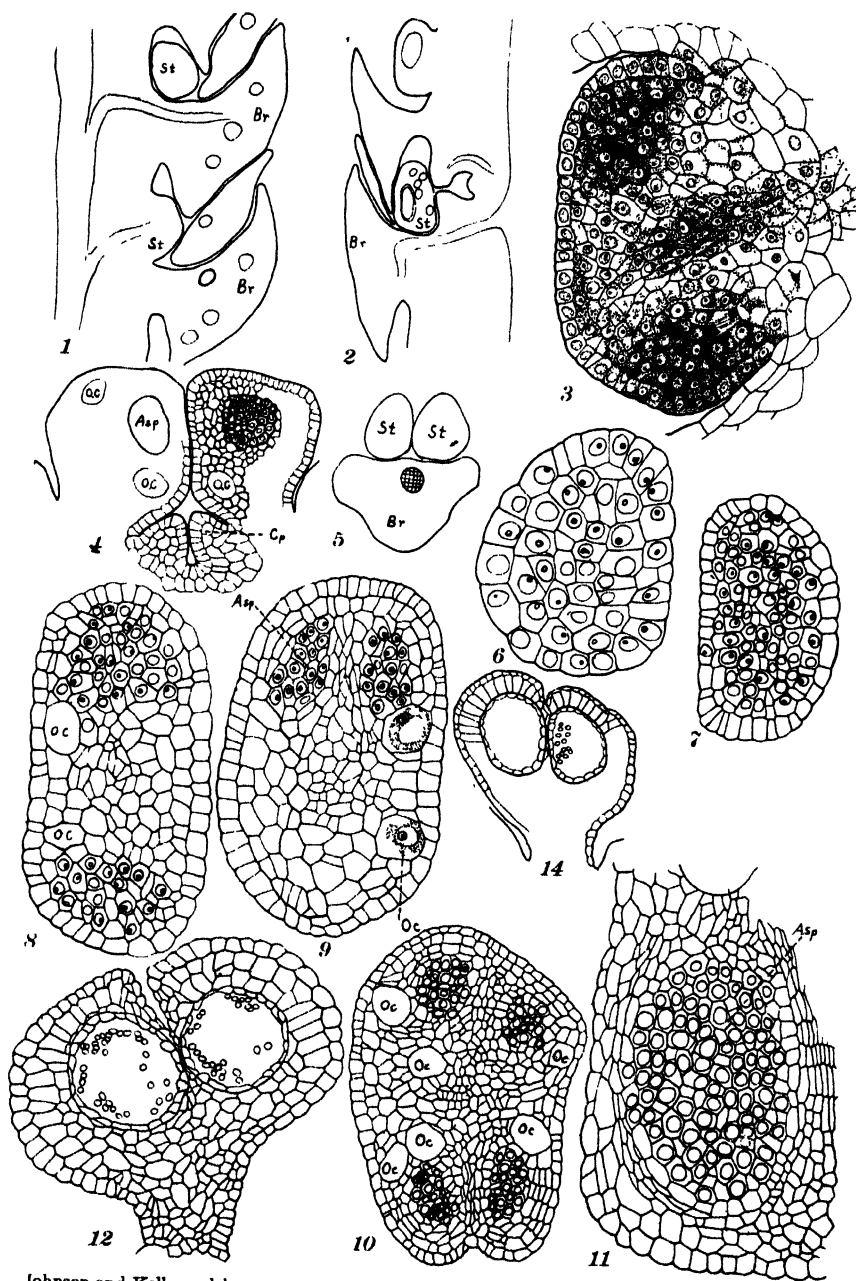
10 Similar section of slightly older stamen, showing two archesporial masses (microsporangia) in each loculus. $\times 210$.

11 A single microsporangium from the stamen shown in fig. 26. $\times 350$.

12 Longitudinal section of a stamen, from a transverse section of a spike, showing microspores, tapetum, wall and line of dehiscence. $\times 150$.

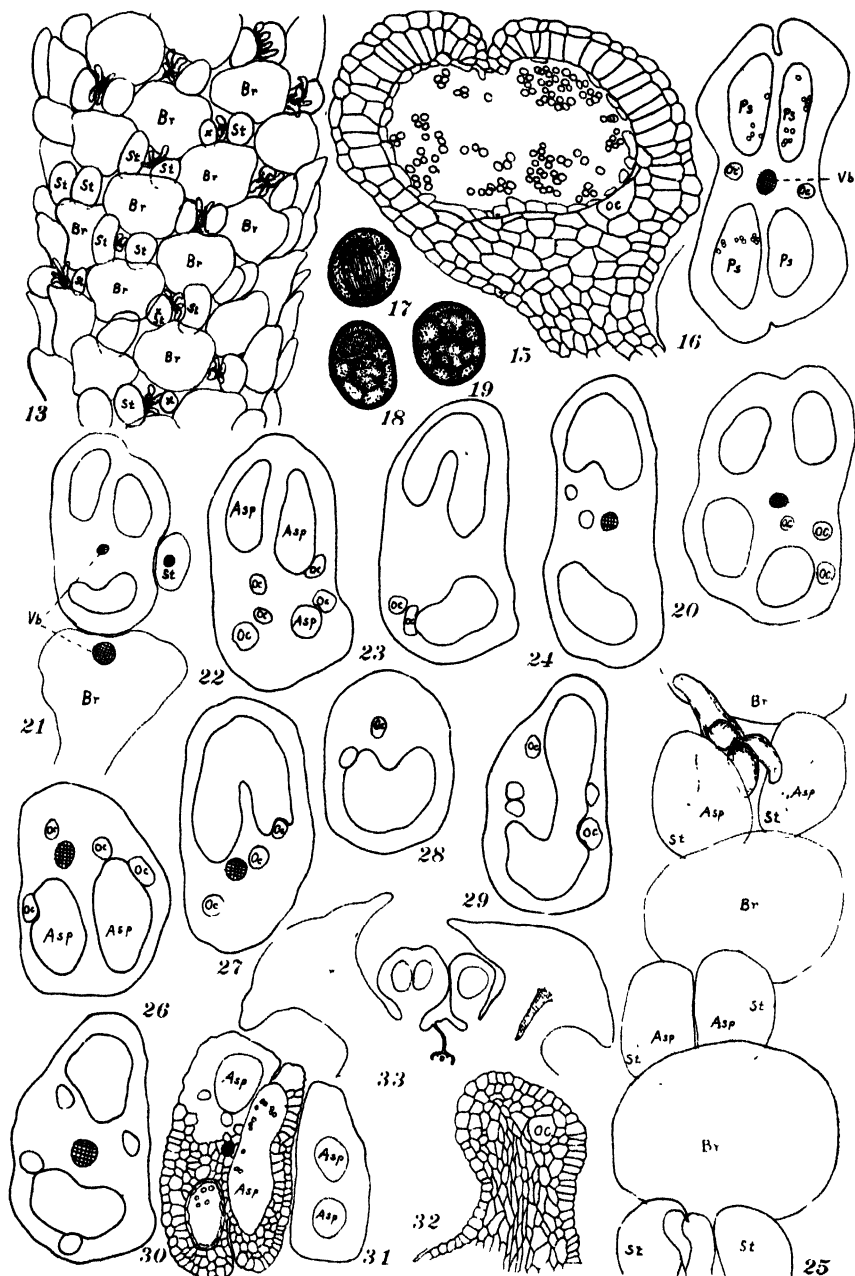
13 Surface view of part of spike, showing overlapping of bracts and the variable number of stamens and stigmas. $\times 10$.

14 Longitudinal section of stamen, from transverse section of spike. $\times 75$.



EXPLANATION OF FIGURES

- 15 Section of the stamen shown in the last figure, several sections removed from the one there shown. $\times 160$.
- 16 Transverse section of a nearly mature stamen with four microsporangia. $\times 75$.
- 17 Section of nearly ripe pollen-grain, showing mitosis of first division. $\times 1000$.
- 18 Section of older, two-celled pollen grain. $\times 1000$.
- 19 Ripe pollen-grain, in which the wall has disappeared. $\times 1000$.
- 20 Transverse section of half-mature stamen with four microsporangia. $\times 100$.
- 21 Transverse section of stamen with three pollen sacs, of a staminodium and of a bract. $\times 100$.
- 22 Transverse section of stamen, showing two sporogenous areas in the upper loculus and one in the lower. $\times 100$.
- 23 Another section of the same stamen, showing the continuity of the sporogenous mass in the upper loculus. $\times 100$.
- 24 Transverse section of stamen showing one sporogenous mass in each loculus. $\times 100$.
- 25 Surface view of spike, showing bracts, stigmas and variety in distribution of the sporogenous masses. $\times 38$.
- 26 Transverse section of stamen with two sporangia in the lower loculus and none in the upper. $\times 100$.
- 27 Transverse section of a stamen with one sporogenous mass in the upper loculus and none in the lower. $\times 100$.
- 28 Transverse section of stamen with one sporogenous mass in the lower loculus and none in the upper. $\times 100$.
- 29 Transverse section of stamen with one continuous sporogenous area. $\times 100$.
- 30 Transverse section of the same anther at a level nearer the base, apparently showing two sporogenous masses. $\times 100$.
- 31 Transverse section of anther showing continuous sporogenous mass across the anterior face of anther, while one sporangium in each loculus remains distinct. $\times 75$.
- 32 Longitudinal section of staminodium, of about same age as the stamens shown in figs. 20-30. $\times 160$.
- 33 Longitudinal section of flower and two bracts, showing relation of these structures and the complete submergence of the ovary in the axis. $\times 40$.



Johnson and Kellner, del.

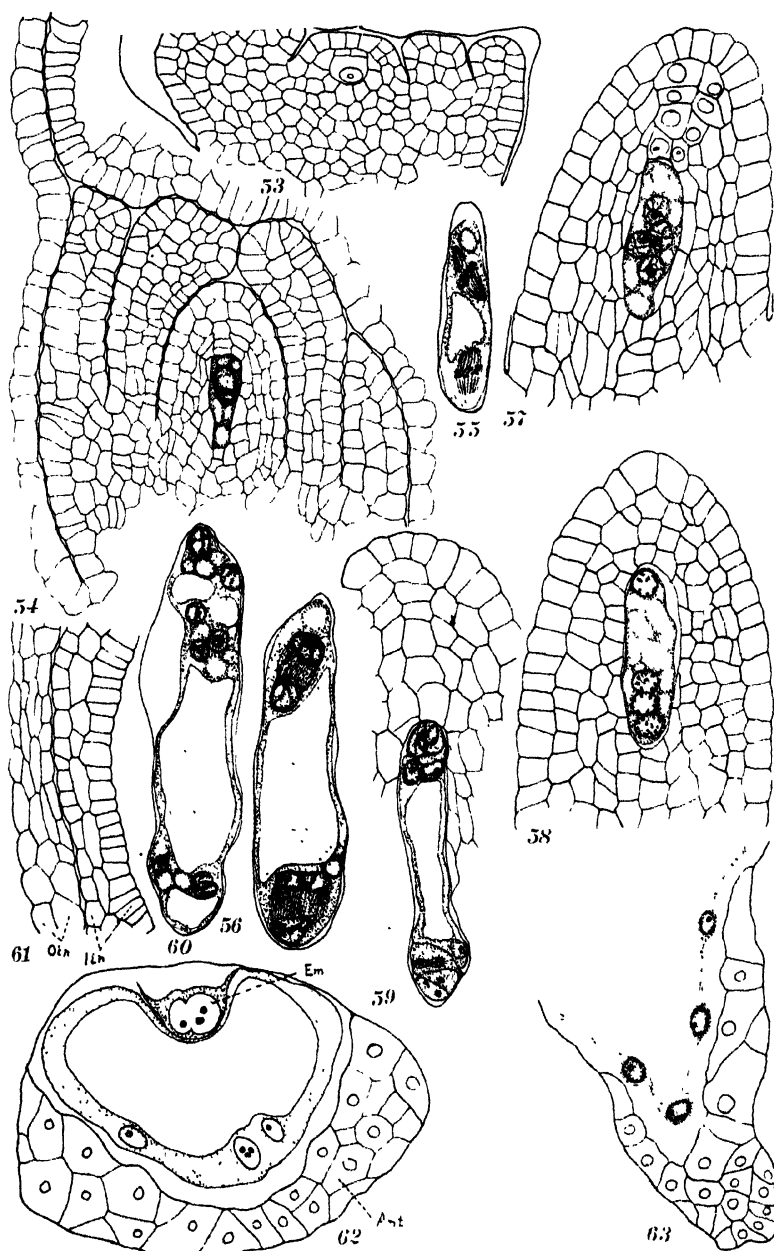
EXPLANATION OF FIGURES

- 34 Longitudinal section of flower with one staminodium. $\times 40$.
- 35 Transverse section of tip of carpels showing three styles. $\times 75$.
- 36 A lower section of the same ovary. $\times 75$.
- 37 Transverse section of another ovary with four styles. $\times 75$.
- 38-43 Series of successively lower transverse sections of an ovule and its integuments, showing the different height of the integument on different sides of the ovule. $\times 160$.
- 44 Longitudinal sections of flower with mature stamens and stigmas and with four-nucleate embryo-sac. $\times 35$.
- 45 Longitudinal section of flower, and two bracts, in which the pollen has been discharged from the single stamen. $\times 35$.
- 46 Longitudinal section of flower with two stamens, well-developed stigmas, but no ovules in the cavity of ovary. $\times 40$.
- 47 Section of another flower like last but with partially aborted styles and stigmas. $\times 35$.
- 48 Section of similar flower but with no ovarian cavity and a mere spine in place of styles. $\times 40$.
- 49 Section of similar (but younger) flower with still more reduced styles. $\times 75$.
- 50 Longitudinal section of flower from male spike, with no trace of carpellary tissues evident. $\times 40$.
- 51 Longitudinal section of flower, from middle of male spike, in which both stamens and carpels are rudimentary. $\times 75$.
- 52 Longitudinal section of young ovary, with ovules showing inner integument. $\times 350$.



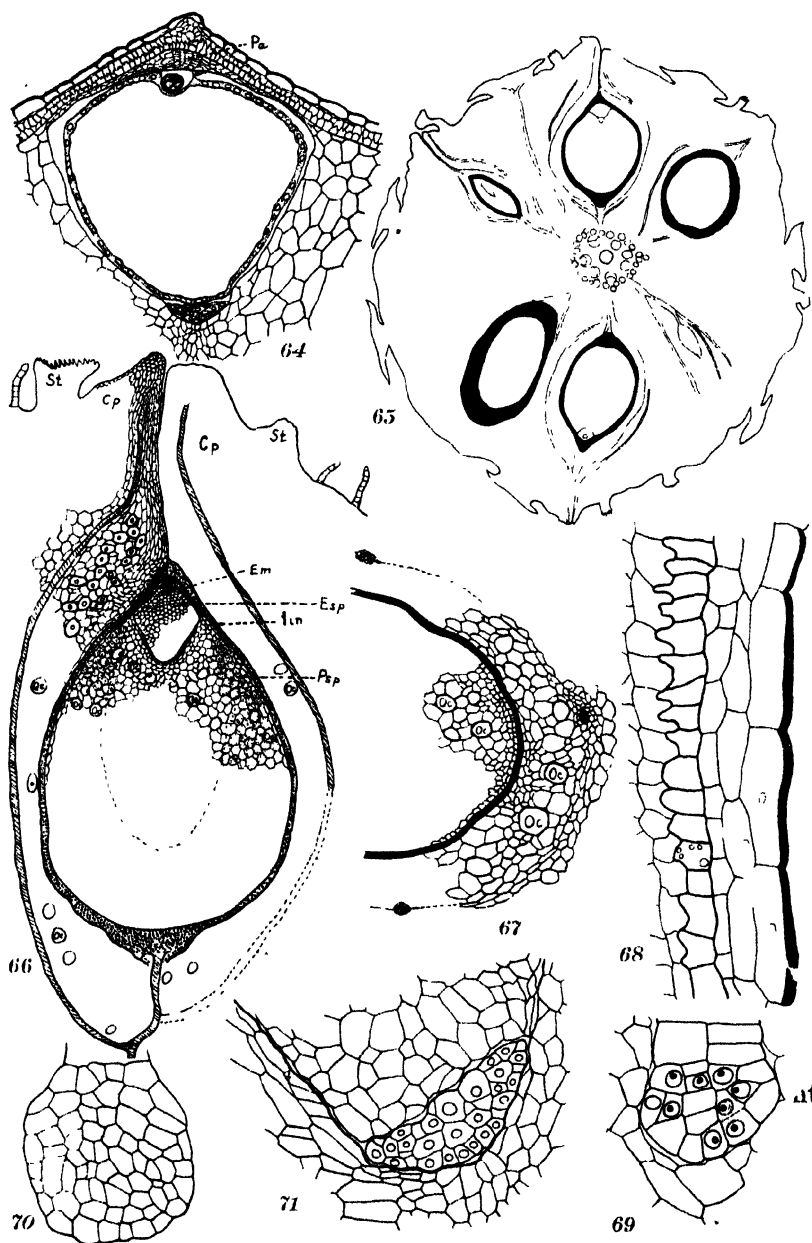
EXPLANATION OF FIGURES

- 53 Longitudinal section of young ovule with two integuments, parietal cell already formed. $\times 350$.
- 54 Longitudinal section of older ovule, with integuments completed and the megaspore mother-cell preparing for its first division. $\times 350$.
- 55 Embryo-sac preparing for the second division, each spindle shows about eighteen chromosomes. $\times 600$.
- 56 Section of a four-nucleate sac, showing a pair of nuclei at each end. $\times 750$.
- 57 Section of a four-nucleate sac, showing the nuclei grouped near the middle. $\times 600$.
- 58 Section of four-nucleate sac with one nucleus at the micropylar end and three at the antipodal end. $\times 600$.
- 59 Longitudinal section of nearly mature sac, in which the polars have not yet moved to the middle. $\times 600$.
- 60 Longitudinal section of nearly mature, somewhat abnormal embryo-sac. $\times 600$.
- 61 Part of longitudinal section of integuments of an ovule with a nearly mature embryo-sac. $\times 350$.
- 62 Longitudinal section of sac, with fertilized egg, free endosperm nuclei, and 25 antipodals in the single section. $\times 170$.
- 63 Part of a similar section, showing endosperm nuclei, and numerous antipodals in a deep pocket at the base of the sac. $\times 180$.



EXPLANATION OF FIGURES

- 64 Part of longitudinal section of seed, showing one-celled embryo, free endosperm nuclei, antipodals, tapetum, inner integument and part of the perisperm. $\times 75$.
- 65 Transverse section of spike, showing number and arrangement of ripe fruits. $\times 10$.
- 66 Longitudinal section of a mature fruit, showing structure of fruit and seed. $\times 40$.
- 67 Part of transverse section of mature fruit. $\times 40$.
- 68 Part of longitudinal section of inner integument, showing structure. $\times 350$.
- 69 Longitudinal section of a half-grown embryo. $\times 350$.
- 70 Similar section of mature embryo. $\times 350$.
- 71 Part of longitudinal section of ripe seed, showing perisperm and persistent antipodal mass. $\times 170$.



A COMPARISON OF THE SENSE-ORGANS IN MEDUSAE OF THE FAMILY PELAGIDAE

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THIRTY-EIGHT FIGURES

The work to be described in the following pages was done at the suggestion and under the supervision of Professor Brooks at Baltimore during the winters of 1888-89 and 1889-90 and during the summer of 1889 in the Fish Commission Laboratory at Woods Hole, where that summer Professor Brooks was director of the Laboratory and I the proud holder of the university table—my first appointment. The manuscript and illustrations were completed and handed to Professor Brooks in November, 1890, with the intention that the paper should form a part of the monograph on the medusae that he then was planning. That book has not been published.

The point of view from which this paper was written is exhibited in the following sentences quoted from the opening paragraph of the manuscript of 1890: "In his *System der Medusen* (p. 504) Haeckel says, 'The genera *Ephyra*, *Palephyra*, *Zonephyra*, *Pelagia*, and *Chrysaora* form five steps in a connected phylogenetic process of development which is repeated at the present time in the ontogeny of *Chrysaora* according to the fundamental law of biogenesis.' As indicated in my preliminary paper (1890), Dr. Brooks has pointed out that *Dactylometra* should be added to the series as the final form, and that, so far as the development

of these genera is known, each genus in the course of its ontogeny recapitulates in each successive stage the condition which remains permanent in each of the lower genera. The lack of a single important break in the series of adult forms is remarkable, and in Palephyra and the yet simpler Ephyra are found structures that show relationship to still more primitive forms." It was my task to discover whether a study of the anatomy and development of the sense-organs would or would not confirm this generalization.

At the present day that point of view appears rather quaintly old-fashioned. Nevertheless it seems worth while to publish the paper because it contains an account of the anatomy and development of these interesting organs that is more complete than any hitherto published, so far as the species herein treated are concerned. And it seems especially appropriate that an article reflecting, as this one does, the thoughts and methods of Professor Brooks at the period of his greatest activity, should appear in the present volume. *

Since this paper was written the conditions of the problem have changed considerably. On the side of greater complexity a new genus, *Kuragea*, has been added by Kishinouye ('02), and on the other hand Vanhöffen ('02) suggests that Haeckel's genera *Ephyra* and *Zonephyra* are not phylogenetic but ontogenetic steps and he unites all three into the single genus *Palephyra*.

Mayer¹ has thrown similar doubt upon the mutual relationships of the genera *Chrysoara*, *Dactylometra*, and *Kuragea*. He thinks that the so-called *Chrysaora* of our coast is nothing more than an imperfectly developed *Dactylometra*. He retains the genus *Chrysaora*, however, for three imperfectly separable species, and my study of the sense-organs seems to indicate that the forms on our coast are really two species, and perhaps represent two separate genera.

Since 1890 our knowledge of the marginal sense-organs of the Scyphomedusae has been increased by Hesse's detailed descrip-

¹ I am greatly indebted to Dr. A. G. Mayer for the gift of a set of the proof sheets of his forthcoming work on the medusae and for permission to quote from them.

tion ('95) of the sense-organs of *Rhizostoma*, in connection with which he refers to special points in the anatomy of these organs in *Cotylorhiza*; by Vanhöffen's ('02) descriptions and figures of the sense-organs of *Atolla*, *Periphylla*, *Sanderia*, and *Dactylometra Africana*; and by my account ('00) of the development of the rhopalia by metamorphosis of the larval tentacles in *Cassiopea*. Vanhöffen's description of the sense-organs of *Dactylometra* is very brief, and neither he nor Hesse mentions the peculiar pitted lateral pockets that I find in the sensory niche of *Chrysaora* and *Dactylometra*, although Hesse finds in *Rhizostoma* a thickened area of sensory epithelium in a similar position, thus confirming Eimer's earlier observation.

In the following pages reference to the work of other investigators will be made as occasion may arise. For a general historical review of the subject the reader is referred to the admirable summaries given by the brothers Hertwig ('78) and von Lendenfeld ('82) and later by Hesse ('95).

The material for this investigation consisted for the most part of preserved specimens furnished by Dr. Brooks including some young stages of *Chrysaora* collected by Professor F. S. Lee. I am indebted to Mr. Austin Cary for some young material of *Dactylometra* collected, I think, at Newport. In view of Mayer's observations it should be noted that when reference is made to *Chrysaora* the form found in the Chesapeake Bay is meant, and the name *Dactylometra* is used for the form found in the open sea.

In the series *Palephyra*, *Pelagia*, *Chrysaora*, *Dactylometra*, and *Kuragea*, as generally understood, we have a series of medusae of similar form and structure. In all the umbrella is relatively flat and is provided at its margin with eight sensory clubs, or rhopalia, four perradial and four interrarial.

These genera may be distinguished briefly as follows. *Palephyra* has 8 tentacles, 16 marginal lappets and in the stomach are four interrarial septal nodes, homologous with the septa of the scyphistoma larva (Bigelow, '00). An account of the possession of these septal nodes *Palephyra* is taken by Vanhöffen ('91) from Haeckel's order *Discomedusae* and placed with the

Periphyllidae in a great group of Scyphomedusae, separate from the group containing the following genera, which lack the nodes.

The genera *Pelagia*, *Chrysaora*, *Dactylometra*, *Kuragea*, and *Sanderia* constitute the family Pelagidae of Gegenbaur (Mayer). *Pelagia* is like *Palephyra* in having 8 tentacles and 16 marginal lappets, but, as has been said, lacks the septal nodes. *Chrysaora* has 24 (3×8) tentacles, 3 between each successive pair of marginal sense-organs, and 32 marginal lappets. *Dactylometra* has 40 (5×8) tentacles, 5 between each pair of sense-organs, and 48 marginal lappets. *Kuragea* ends the series of progressive steps in complexity in this line with 56 (7×8) tentacles and 64 marginal lappets. *Sanderia* is an aberrant form in which all the marginal parts of *Pelagia* have been doubled, thus having 16 marginal sense organs, and likewise 16 tentacles separated by 32 left marginal lappets.

Unfortunately nothing is known in regard to the structure of the sense-organs of *Palephyra*, and there is some doubt as to how far this genus should be regarded as representing an ancestral type of the group of Medusae classed by Haeckel in the order Discomedusae.

The present account of the sense-organs deals with those of *Pelagia cyanella* Péron et Lesueur, the *Chrysaora*-like form of the Chesapeake Bay, and *Dactylometra quinquecirrha* L. Agassiz. First, three successive stages in the development of the sense-organ of *Chrysaora* will be described, leading to a description of the adult condition. This will be followed by a comparison of the adult sense-organs of *Pelagia* with the *Pelagia*-stage of *Chrysaora* and with the adult. Finally these structures in the adult *Dactylometra* and in some stages of its development will be compared with the corresponding stages in the other species.

THE EPHYRA STAGE IN CHRYSAORA

The youngest specimen of Chrysaora that came under my observation was an ephyra larva in which the tentacles were just beginning to bud. The eight arms of the ephyra are tipped each by two lobes and there is suspended from the under side, a little proximal to the notch between the lobes, a sense organ called "Sinneskolbe" or "Randkörper" by most German authors, the name *rhopalium* given to it by Haeckel is, however, the most convenient for us and is the one I shall use. Seen from below this appears to be a short club-shaped structure lying horizontally on the under side of the umbrella, fig. 21. In describing the adult form the Hertwigs very aptly compare it in shape to a bent finger. This applies equally as well here (compare figs. 2 and 22). The part by which the rhopalium is attached is perpendicular to the oral surface of the umbrella. The other part extends at right angles to the first away from the mouth and in its distal end is a conspicuous cluster of crystals, fig. 21. The tip of the sense-organ reaches to the edge of the notch between the lobes. The lobe of the stomach that penetrates each arm has about one-third the width of the latter. At the base of the rhopalium it narrows into a small tube, fig. 22, *r.c.*, which bends downward and extends into the rhopalium as far as or a little beyond the angle, where it ends blindly. I shall speak of this tube as the rhopalial canal. There is no extension of the gastric pouch into the lobes of the arm, as has been shown already in the figures given by Agassiz, Claus, and the Hertwigs, and this seems to be generally true of this larval stage.

The endodermal lamella, fig. 25, *e.l.*, is plainly visible along the side of the gastric pouch, which is more or less triangular in cross section. The lamella extends from the lower angles of the pouch obliquely downward and soon joins the ectoderm and does not extend to the margin of the arm.

The endoderm gradually becomes thickened towards the sense-organ until where the rhopalial canal bends downward the cells are deeper than broad. The distal end of the rhopalium is completely filled with large thin-walled endoderm cells, each of which

secretes a concretion in its interior, fig. 22. These concretions are soluble in weak acids and vary in size and shape, but are generally prisms not more than twice as broad as long with conical ends and they seem to contain a core of organic matter. There is no sharp line of distinction between the cells which produce these bodies and the other endoderm cells of the rhopalium, and for Von Lendenfeld to call this cluster of cells the "visceral mesoderm" is rather stretching analogies.

The general ectoderm of the body is composed of very much flattened cells a little thickened in the position of the nucleus. In spots over the surface, the cells are much thicker so that they appear square in vertical section. These are the young batteries of nettle cells, figs. 22 and 26, *b*. At the base of the rhopalium the ectoderm is abruptly very much thickened and forms a deep covering to all but the most distal part of it, fig. 22. In this thickened part the cell walls are hardly to be distinguished; the nuclei are thickly crowded in two or more irregular rows and between this layer of cells and the thin supporting membrane there appears a thin layer of nerve fibers. This thick ectoderm thins out at the distal extremity of the rhopalium until in my preparations it cannot be recognized, but it is undoubtedly there as a very thin membrane. The thin supporting membrane which everywhere separates the ectoderm from the endoderm is a continuation of the general mesogloea, which is structureless except for a few fibers running through it. The sense-organ of *Chrysaora* at this stage then, is quite simple; there is no trace of an olfactory groove, and there are no other organs accessory to the rhopalium.

THE PELAGIA STAGE IN *CHRYSAORA*

After the ephyra the next youngest stage of *Chrysaora* that I have studied is a larva six millimeters in diameter. The beginnings of the gonadia have not yet appeared, but the animal has eight tentacles, hollow at least for a long distance. The pendant oesophagus is rather short, but there are four oral lobes which when spread out extend more than halfway to the edge of the disk, and there are two endodermal pockets into each of the mar-

ginal lappets. This then may be regarded as in the Pelagia stage.

The rhopalium at this stage is twice as large as in the preceding one and the distal horizontal part is longer in proportion to the proximal vertical part, fig. 26. It lies in a small cavity generally known as the sensory niche (*Sinnesnische*) *s. n.*, figs. 26 and 28, which is not at all developed in the ephyra. This is bounded above by the hood, figs. 26, 27, and 28, *h*, laterally, by the sides of the marginal lappets; and proximally, by that part of the subumbrellal wall that rises to pass into the hood. The niche becomes gradually shallower proximally; distally and below, the niche is open, except that the free edges of the marginal lappets are folded together so as to convert the part of the niche immediately surrounding the rhopalium into a tube such as is figured by the Hertwigs. There is a low, flat ridge, figs. 26 and 30, *r. r.*, extending along the slanting roof of the niche from the proximal wall outward about halfway to the edge of the hood. The rhopalium hangs from this ridge and its base covers more than half of it. The ridge contains the rhopalial canal which sends a little conical diverticulum into a small part of the ridge that extends beyond the base of the rhopalium, fig. 26, *r. c.* The canal penetrates the rhopalium for about half of its length, considerably farther than in the ephyra.

The endoderm at the mouth of the rhopalial canal begins to thicken and farther inward becomes columnar. These columnar cells distally fill the whole lumen of the tube and they grade off into the larger otolith cells of the extremity. These are about the same size as in the ephyra, but are more numerous, and as in the ephyra the endoderm is separated from the ectoderm throughout the rhopalium by only a thin supporting membrane.

The general ectoderm of the body, including that lining the sensory niche, is flat as in the ephyra, but the thickened spots (*b*) are more developed and the nettle cells are clearly differentiated in them. The ectoderm on the rhopalium is twice as thick as in the ephyra and grades distally, as in that stage, into a thin membrane covering the outer end. The gradation is more abrupt on the upper than on the lower side.

Around the base of the rhopalium this very much thickened ectoderm passes into the general flat epithelium of the body, except at two points. These are at sides of the rhopalial ridge, fig. 30, and here the rhopalial ectoderm is continuous with two folds of thickened ectoderm which run on each side of the ridge centrally to the proximal wall of the niche, fig. 31.

The sensory epithelium of the rhopalium, figs. 26 and 28, is of the same character as that in the ephyra and the cells are provided with comparatively long cilia. In this stage the layer of nerve fibers is very well marked; in the deepest part it is as thick as the cellular layer. This thickened portion of the nerve fiber layer describes a U-shaped figure. The loop of the U lies on the upper side of the rhopalium in the most distal part of the nerve layer, fig. 28, and the limbs of the U extend along the upper part of each side of the rhopalium and each one is continuous with a thin layer of nerve fibers underlying the before-mentioned lateral thickenings of the rhopalial ridge. The ectoderm in these latter areas is composed of very small cells with very long cilia. In section the layer of cells seems to be composed of a mass of crowded nuclei. It is probably a single layer of cuboidal or slightly elongated cells and it is folded so that there is an *invaginated groove* at each side of the rhopalial ridge which extends to the proximal end of this thickened ectoderm on the wall of the niche. The groove runs along the upper edge of the layer and in addition to it there are a number of shallow *secondary pits*. On the proximal wall of the niche the lateral folds lie in the plane of the endodermal lamella and are apparently in contact with it, fig. 32. All along the rhopalial ridge there is a line opposite the bottom of the principal fold and continuous with the endodermal lamella in which the endoderm and ectoderm are in contact. This lamella not only connects the endoderm of adjacent gastric pouches but also connects the endoderm with the ectoderm all around the margin of the umbrella, except where the jelly is too thin to permit it, and it is remarkable that in all forms that I have studied there is some point where this lamella comes into contact with the nerve-fiber layer.

THE BEGINNING OF THE ADULT CONDITION

With the appearance of the tentacles characteristic of the *Chrysaora*, or shortly after, the foundations of the other adult structures are established. The description of this initial stage will be taken from a specimen 10 mm. in diameter, in which the second set of tentacles reached to about the tips of the lappets. Although a good deal larger, the rhopalium and adjacent parts are about in the same proportion as in the preceding stage, compare figs. 26 and 33.

The endoderm cells of the rhopalium are more narrowly columnar than in the last stage and the lumen of the canal reaches to the concretion-forming cells. The transition from the columnar cells to these is rather abrupt. These cells have increased in size and number and the whole mass of concretions is nearly spherical, fig. 33.

The ciliated ectoderm of the rhopalium has increased still more in thickness and the nerve-fiber layer is still more marked than in the last stage. There has been but little change in the folds at the sides of the rhopalial ridge, yet it is in the ectoderm that the most important changes have taken place. In the first place, the epithelium of the lower surface of the rhopalial ridge, which was slightly thickened in the pelagia stage, is now very much more thickened, the cells becoming cuboidal or almost columnar, while the epithelium of the roof of the sensory niche adjoining the rhopalial ridge has suffered a similar change.

But the chief step in advance is the formation of the "olfactory groove," or, as von Lendenfeld prefers to call it, "the dorsal sensory groove." This is a shallow saucer-shaped depression in the exumbral surface of the hood just above the base of the rhopalium, figs. 33 and 34, *s.g.* In this groove the ectoderm is considerably thickened, being composed of a single layer of columnar cells. These cells are deepest in the deepest part of the groove. In specimens of about the same size as the one just described but in which the evaginations that are to form the second set of tentacles are not longer than they are broad, a shallow dorsal groove occurs, but it is clothed simply with the ordinary flat epithelium.

THE ADULT CHRYSAORA

Turning now to the fully formed adult, figs. 2 to 10 (fig. 1 is not fully adult), we find no structure not represented in the stage just described, but there are marked changes in form and proportion and there is much greater histological differentiation.

The size of the animal and of all of its parts has greatly increased, the general ectoderm and endoderm have become more marked, the cells being thicker in proportion to their width, the nettle cells and gland cells are fully developed, and the general topography in the region of the sense-organ is very much heightened, fig. 2.

On looking down upon the upper side of the umbrella, one notices in the hood covering each rhopalium an elliptical area free from the nettle batteries that now form thickly set mounds over the rest of the surface. These elliptical areas are the dorsal sensory grooves, an early stage of which has just been described. Each groove on closer examination is seen to be now a funnel-shaped cavity, the apex of the funnel extending deep into the mesogloea to a point opposite the base of the rhopalium, fig. 6.

At the edges of the groove the common cuboidal epithelium of the exumbrella grades into a deep columnar epithelium that lines the groove. The cells of the epithelium are many times deeper than they are wide and are ciliated, and at the base of the layer next to the mesogloea there is a thin stratum of nerve fibers. This epithelium is probably of that kind of sensory epithelium common to jelly fish which has been carefully described and figured by Eimer ('77), Claus ('77), the Hertwigs ('78), and Schewiakoff ('89). There are present some mucous gland cells like those found by Wilson ('88) in *Manicina*. The mucus granules stain so deeply with haematoxylin that the mass of mucus might easily be mistaken for a nucleus, but they do not stain with carmine. The surface of the groove, being perfectly even, shows no trace of such complications as are described by Claus in *Aurelia*, by von Lendenfeld in *Cyanea* and *Crambessa*, and by Hesse ('95) in *Rhizostoma*.

If the animal now be looked at from the under side, the most conspicuous part of the sensory apparatus is the rhopalium, figs. 1, 2, and 10, *r.*, which has the same shape that it had in the previous stage, that of a thick bent finger, and it has not increased in size in proportion to the surrounding parts. The sensory niche in which the rhopalium lies is very much deeper than in the previous stage and the free edges of the marginal lobes are approximated so as to form a tube which extends from about opposite the base of the rhopalium outward to the edge of the hood. The rhopalium is attached as before to a ridge which extends centrally along the arched roof of the niche from a point just distal to the base of the rhopalium to the proximal wall of the niche. But now the base of the rhopalium covers but a very small part of the ridge near its distal extremity, and the proximal part is relatively, as well as actually, very much larger than before, figs. 1, 2, and 10. The ridge gradually becomes wider and thicker as it recedes from the rhopalium, figs. 1 and 2, and 5 to 8. Its under surface is convex and it is grooved on its sides. These lateral grooves are continued into two pocket-like cavities which lie on each side of the ridge in the upper proximal wall of the niche, figs. 1, 2, and 8. The rhopalial canal becomes gradually narrower from its mouth outward to the base of the rhopalium, it is slightly dilated in the proximal part of the latter and extends some distance into the distal part where it ends as a narrow pocket. In a section of the mass of concretions it appears as a narrow vertical slit, figs. 1 and 3. The canal sends off a very small cone-shaped diverticulum into the hood above the rhopalium.

With this heightening of the general topography comes a corresponding increase in the importance of the various histological features. The thickening of the ectoderm, which in the last stage extended a short distance around the base of the rhopalium, has now spread so as to cover the whole surface of the sensory niche, including all but a small part at the base of the rhopalium of the convex under surface of the rhopalial ridge, and has come to form apparently a sensory epithelium. It extends in all directions from the base of the rhopalium, laterally nearly as far as

the bases of the marginal lappets, distally as far as the extremity of the hood, and proximally to the edge of the subumbral muscle layer, a very small part of which comes within the hollow of the niche. The thickness of this layer of cells varies somewhat.

The ectoderm layer is thickest on the proximal wall of the niche and two thickened areas extend outward along the sides of the marginal lappets midway between the rhopalial ridge and the free edges of the lappets, gradually thinning out at a point beyond the margin of the hood. At the edge of the muscle layer this epithelium of the niche passes rather abruptly into the cells covering the muscles, and the columnar epithelium extends some distance outward between the edge of the muscle layer and the endodermal lamella on the subumbral wall of the marginal lappets, fig. 3. The free edges of the marginal lobes are covered by a simple slightly flattened epithelium. The sensory epithelium of the niche grades gradually into this. Surrounding the base of the rhopalium on its distal and lateral sides, the epithelium is only about half as thick as it is in the deepest part of the niche.

Where the sensory epithelium of the niche is deepest the cells are of about the same proportion as in the dorsal sensory groove, perhaps somewhat longer. These cells as seen in sections are long and columnar, each with a nucleus in the lower third. Below the nucleus the protoplasm is clear, above it, it is granular, and in the upper third of the cell there is often the characteristic structure of a mucous cell.

The mucous cells, which are here very abundant, are also found in the general ectoderm and endoderm, and particularly in the dorsal sensory groove (as already stated) and in the endoderm of the rhopalial canal. In each place the goblet cell is of the same length as the adjoining cells and everywhere, except in the dorsal groove and the sensory niche, the globule of mucus nearly fills the whole cell. These cells seem, however, to be absent from the epithelium of the under side of the rhopalial ridge and from that lining the niche immediately proximal to it, so that in a longitudinal section through the rhopalium they are not seen, fig. 12. Scattered nettle cells are found also in the epithelium of

the niche and rarely I have come across one in a section of the rhopalial canal.

Cyanea annaskala presents, according to von Lendenfeld, a precisely similar arrangement of the epithelium of the niche, except that in this species there is a peculiar sensory apparatus on the dilated proximal part of the rhopalial ridge which can be compared to nothing in the Pelagidae. Von Lendenfeld has also found in *Crambessa* a pair of rounded thickenings containing sub-epithelial ganglion cells which correspond in position with the areas of deep columnar epithelium that I have described as extending outward along the sides of the niche. Von Lendenfeld homologizes these with the cone-shaped thickenings found by Claus in *Aurelia* and *Chrysaora*, but they seem to be something entirely different, as will appear later.

The cellular covering of the extremity of the rhopalium can now be clearly seen to be a simple, slightly flattened epithelium. It grades, more gradually on the lower than on the upper side, into the layer of columnar cells and nerve fibers which covers the main part of the rhopalium, fig. 10. This is similar to the sensory epithelium that has been found by Eimer, Claus, Schäfer, and Schewiakoff in *Aurelia*, by the Hertwigs in *Pelagia*, by Schewiakoff in *Carybdea*, by Hesse in *Rhizostoma* and by Verhöffen in several species. It is a deep ciliated epithelium of slender cells with the nuclei placed irregularly in several rows. There are numerous straight fibers extending from the cellular layer through the thick felted nerve-fiber layer to the supporting membrane. These are processes of cells described in Schewiakoff's paper ('89) and regarded by him as supporting cells.

On the distal side of the base of the rhopalium its epithelium passes into the ordinary epithelium of the niche. On the proximal side it grades into a peculiar epithelium that forms a structure of which we found the rudiments in the *Pelagia*-stage, fig. 32. This epithelium, like that on the rhopalium, overlies a layer of nerve fibers. It is at this stage clearly a single layer of short cuboidal cells provided with very long cilia, fig. 11. The nuclei are of the same size and appearance as in the sensory epithelium of the rhopalium and nearly fill the cell. The layer is very much

folded and pitted. The mesogloea, however, takes no part in this folding, but the nerve fibers extend outward between the cells which line adjacent pits. This folded epithelium covers a small part of the lower surface of the rhopalial ridge (fig. 10) and extends along the lateral grooves into the pockets that have been mentioned as being sunk into the mesogloea at the sides of the mouth of the rhopalial canal. The pits are thickly set and in the lateral pockets they are very deep and fill nearly the whole of the pocket. The lumen of each pocket opens into the fundus of the niche on one side of the ridge, figs. 2 and 11. The pocket is much wider horizontally than vertically and is deeper than the proximal wall of the niche, so that its apex occupies a small prominence in the roof of the gastric pouch at the sides of the mouth of the rhopalial canal, figs. 8 and 9.

This pitted ectoderm is separated from the endoderm by only a thin supporting membrane for the whole distance from the base of the rhopalium to the apex of the pocket. From this point the mesogloea thickens and then the endodermal lamella appears and continues in contact with the pocket around to its adradial extremity, fig. 8. I cannot, however, discover any protoplasmic connection between the cells of the lamella and those of the pocket.

The nerve-fiber layer which lines the pocket and underlies the whole of the pitted epithelium is of the same character as the nerve layer of the rhopalium, being a felted mass of extremely fine fibers, and is directly continuous at the base of the rhopalium (fig. 11) with the limbs of the U-shaped thickening which I have described in the previous stages. This thickening is now very prominent on the rhopalium and the membrane beneath it is thickened for its support, fig. 5.

The pitted epithelium of the lateral grooves and pockets is, of course, derived from the lateral folds of ectoderm of the previous stage. Structures of the same kind were probably seen by Eimer and certainly were by Claus in *Cyanea* and in *Aurelia*, respectively. Eimer ('77) says that in *Cyanea* the ectoderm surrounding the rhopalium forms numerous conical ingrowths. Claus ('77) in speaking of *Aurelia* says that "there is found at the base

of the marginal body in the sensory niche a pair of cone-shaped swollen thickenings of the ectoderm which enclose under the epithelium a thick layer of ganglion cells and nerve fibers." He says, moreover, that the nervous system in *Chrysaora* has the same general structure as in *Aurelia*. These statements apparently refer to the structures that I have described, but fail to give a correct idea of them. As already stated, von Lendenfeld found in *Crambessa* two prominences on the sides of the niche which he homologizes with the cone-shaped thickenings of Claus. But as he distinctly says that they are not invaginations, they must be quite different from the organs to which I suppose Claus alludes. Nothing need be added concerning the histology of the endodermal parts of the rhopalium at this stage except that the cells have increased in depth.

THE ADULT PELAGIA

The brothers Hertwig have given in their work on the nervous system and sense-organs of the medusae ('78 a, p. 109) a very clear account of the position and structure of the rhopalia in *Pelagia noctiluca* (Pér. Les.) and have described their development from the ephyra stage.

This agrees in the main with what I have said of the rhopalium of *Chrysaora* except that the rhopalial canal in the adult does not penetrate the mass of concretions. The ephyra of *Pelagia* agrees in every essential particular with the same stage in *Chrysaora*. As in *Chrysaora* at this stage, the gastric diverticulum does not penetrate the rhopalium but its interior is filled with endoderm cells, and the rhopalium is not surrounded by a sensory niche. The most important events which take place in the development from this to the adult stage are, according to the Hertwigs, the hollowing out of the rhopalium, an increase in the number of concretions, and the formation of the sensory niche by the outgrowth of the hood and free edges of the marginal lobes.

I have studied only the adult sense-organ in *Pelagia cyanella* (Pér. Les.) and can confirm the description of the adult given by

the Hertwigs, so far as it goes. I have noticed additional features which, however, may be peculiar to the species studied.

On the upper surface of the hood immediately above the base of the rhopalium there is a funnel-shaped dorsal sensory groove, fig. 13, *s.g.* It differs from the groove in *Chrysaora* in being much smaller and in sinking into the mesogloea not more than half so far. Hesse ('95) confirms Eimer's statement that a similar groove is to be found in *P. noctiluca*, but found no sensory epithelium in it.

While, as stated by the Hertwigs, the general surface of the niche is covered by the common ectoderm of the body, I find on the rhopalial ridge, which is short and low, fig. 15, a peculiar epithelium which distally passes into the ectoderm of the rhopalium and proximally extends along the wall of the niche towards the muscle band. My material is not sufficient for me to make out very clearly the structure of this layer. It is twice the height of the ordinary epithelium. The outer part of the layer is made up of the thicker parts of the cells, which stain deeply, while the inner portion seems to be composed of processes running from the cells to the surface of the mesogloea. There seems to be a loose network of nerve fibers intermingled with these processes. At any rate, my preparations show very clearly a number of large ganglion cells scattered through this layer just below the deeply stained part, fig. 16, *g.* When I speak of these cells as large, I mean that they are many times larger than the ordinary epithelium cells. They are provided with comparatively large nuclei and are apparently bipolar, the stout processes running parallel to the rhopalium. Cells of this kind similarly situated have been found by Hesse ('95) in *Rhizostoma*, and, as he says, probably constitute a nerve center in each sensory niche.

In the thick layer of nerve fibers on the basal portion of the rhopalium there is a cluster of nuclei like those in the sensory epithelium above them, fig. 14, *n.* These probably belong to very small ganglion cells. At each side of the ridge at the base of the rhopalium the layer of nerve fibers comes into contact with the endodermal lamella, and the latter, which otherwise has the same relative position as in *Chrysaora*, may be traced in this species

along each side of the rhopalial canal nearly to the tip of the diverticulum into the hood, fig. 14. Aside from the rhopalium and the band of peculiar epithelium and nerve fibers there is no other sensory apparatus in the niche.

The sense-organs in *Pelagia* are then much simpler than in the adult *Chrysaora*, but they have many points of resemblance to the *Pelagia*-stage in the young *Chrysaora*. In both the sensory niche, while well developed, is not so prominent as in the adult *Chrysaora* and the greater part of it is lined with the ordinary surface epithelium. It is probable that in the larval as well as the adult form there is a thickening and a differentiation of the epithelium upon the lower surface of the rhopalial ridge. The latter in both is of about the same proportion, being short and but slightly raised, and in both the rhopalial canal extends only to the mass of concretions and does not penetrate it. The shape of the rhopalium in *Pelagia* differs from its shape in the young *Chrysaora*, as well as in the adult, in that the part which contains the concretions has a considerably smaller circumference than the part covered by the sensory epithelium, and the concretions themselves are longer in *Pelagia* in proportion to their width. But the important points in which *Pelagia* differs from the corresponding stage in the development of *Chrysaora* are two: the presence of the dorsal sensory groove, and the absence of any fold of the ectoderm at the side of the rhopalial ridge. Compare figs. 15 and 31. It will be noticed, however, that the line along which the endodermal lamella touches the ectoderm at the sides of the rhopalial ridge in *Pelagia* (fig. 14, *e.l.*) coincides exactly with the position of the deepest part of the lateral folds in the *Pelagia*-like larva of *Chrysaora*.

THE ADULT DACTYLOMETRA

The sense organs of *Dactylometra quinquecirrha* differ from those in *Chrysaora*, as we would expect, in the opposite direction from *Pelagia*. While that genus lacks some of the features of *Chrysaora* and has a simpler sensory apparatus, the adult *Dactylometra* possesses all the characteristics of *Chrysaora* in an exaggerated degree, figs. 17 to 20.

The rhopalium is of about the same shape and size as in *Chrysaora*. It is clothed with the same kind of sensory epithelium overlying a layer of nerve fibers as thick as the layer of cells or thicker in the U-shaped areas. The supporting membrane is thickened under this area and the rhopalial canal extends into the mass of cells that contain the concretions. In short, the rhopalia in the two species are alike in every particular. (It is only in *Pelagia* that I find any trace of ganglion cells in the nerve-fiber layer of the rhopalium.) The parts surrounding the rhopalium are, however, much larger than in *Chrysaora*. The mesogloea of the hood is very much thicker and the dorsal sensory groove is proportionally deeper. It is a little longer than in *Chrysaora* but no wider, figs. 17 and 18. The sensory niche is also deeper, the lateral pockets are larger, and the rhopalial ridge is more prominent.

It is in the latter that we find the most characteristic differences between *Chrysaora* and *Dactylometra*. Immediately at the base of the rhopalium the ridge is covered by a shallow pitted epithelium with its layer of nerve fibers, as in *Chrysaora*, fig. 18. Proximally this soon becomes confined to the sides of the ridge while the area between is clothed with a single layer of small cuboidal cells. At about half the length of the ridge two small elevations appear, one on each side of this simple epithelium, fig. 19. The concave area between them gradually widens until it joins the proximal wall of the niche. The pitted epithelium covers a much greater part of the surface of the ridge and is much more highly developed than in *Chrysaora*. It gradually spreads out on to the roof of the niche and its pits become deeper and more numerous as it recedes from the rhopalium. At its outer edges the change into the ordinary epithelium of the niche is quite abrupt. A comparison of figs. 7 and 19 will show these characteristic differences in the two species. With the increase in size of the lateral pockets there is an increase in the depth and number of the epithelial pits. These are branched and closely crowded so that they fill the whole pocket, obliterating its lumen, and the orifices of the pits open directly to the exterior at its mouth; not well shown in fig. 20.

Except the endodermal lamella, there are no cellular elements imbedded in the mesogloea in the neighborhood of the sense-organ in either of the three species under consideration. There are many fibers in the mesogloea, especially in *Dactylometra*, but they are apparently only connective tissue fibers serving to give firmness or elasticity to the jelly and there is no evidence that they have any connection with the nervous system. Schäfer and Schewiakoff both speak of the connection between the layer of nerve fibers in the dorsal sensory groove and the nerve fibers in the niche, and Hesse finds in *Rhizostoma* fibers extending from the fundus of the dorsal sensory groove to the endoderm of the rhopalial canal, and he regards these as nerve fibers, in spite of the fact that they would not stain with methylene blue or gold chloride. If there is such a connection in the *Pelagidae* it is not through the mesogloea. In fact, the mesogloea immediately surrounding the sensory groove in *Dactylometra* seems to be perfectly structureless, although the fibers are so well developed in other parts of it.

SOME LARVAL STAGES OF DACTYLOMETRA

The questions that now remain to be answered in this paper concern the stages in the development of *Dactylometra*. Unfortunately my material for this purpose is very scant.

The youngest of some larvae taken near Newport that I suppose to be *Dactylometra* is a little less than 2 mm. broad. It has four gastric filaments, the tentacles are just budding and the tentacular pouches of the stomach are about half as long as the rhopalial ones, while from the latter the marginal pockets are beginning to grow into the ephyra lobes. This specimen may, therefore, be regarded as on the border line between the Ephyra- and the Paleephyra-stage. Sections of the rhopalium, fig. 35, show little differences between this and the slightly earlier stage of *Chrysaora* that I have already described. The constriction at the end of the rhopalial canal is less marked and the distal part of the rhopalium may be relatively a little longer.

In a little older one of these specimens the rhopalium, fig. 36, is of the same character as in the *Pelagia* stage of *Chrysaora* but there is only the merest rudiment, if any trace at all, of the lateral ectodermal thickenings. It is rather difficult to compare this larva with any of Haeckel's genera. It is 3 mm. in diameter, has a simple quadrate mouth, eight gastric filaments, one in each pair being shorter than the other, and the tentacular pouches are a little shorter than the rhopalial ones. The pockets into the marginal lobes from the rhopalial gastric pouches are hardly large enough for a *Palephyra* and the pockets from the tentacular pouches show an approach to a *Zonephyra*-stage. The short tentacles like the buds in the younger stage are solid for the greater part of their length. This is the specimen that I spoke of in my preliminary paper as one just passing into the *Pelagia* stage.

I have no specimens of *Dactylometra* in the *Pelagia*-stage; all of my remaining larvae have acquired the rudiments of the second set of tentacles, and are therefore in the *Chrysaora*-stage. It is evident that, as in *Chrysaora*, the first trace of the dorsal sensory groove appears with the second set of tentacles. In a specimen 18 mm. in diameter in which the tentacles of the second set are about 3 mm. long, the sensory groove may be seen as a flat disk of cuboidal or columnar cells lying directly above the base of the rhopalium, fig. 37. The sensory niche is broad and shallow and is lined with the ordinary flat epithelium of the body. The rhopalium shows all the characters of that organ in the adult *Chrysaora*; the rhopalial canal penetrates the distal part in the same way, the layer of nerve fibers and the supporting membrane show the same thickenings and in my sections Schewiakoff's supporting cells can be seen very clearly. The rhopalial ridge is very short and is completely covered with the thick pitted epithelium, fig. 37; this is continued into the relatively very large lateral ectodermal pockets and lines of the floor each pocket and fills its inner half, fig. 38. The roof of the pocket is lined with the ordinary flat epithelium of the niche. At the edge of the pockets the epithelial pits are wide and comparatively shallow, evidently in the process of formation; the layer

of nerve fibers is, however, most noticeable at this point. A thickening of this layer seems to run along the under lip of the pocket. The tips of the pockets extend each one into a prominence on the dorsal wall of the gastric pouch at the side of the rhopalial canal, just as in the adult *Chrysaora*.

In a specimen about 26 mm. in diameter in which the tentacles of the second set are over a centimeter long the dorsal groove is a saucer-shaped depression lined in its deeper parts by a distinctly columnar epithelium. The sensory niche is also deeper, the rhopalial ridge longer, and the lateral pockets more nearly filled with the pitted epithelium, which in turn has a much less embryonic appearance than in the younger specimen.

These specimens should be compared with young *Chrysaoras* of about the same size (fig. 1) rather than with the younger ones (figs. 33 and 34) that I have described as in the beginning of the adult stage. A *Chrysaora* 25 mm. in diameter has its gonadia formed, although they are quite small, and is otherwise intermediate between the beginning of the adult condition and its completion. In the young *Chrysaora* of 25 mm. (fig. 1) the thickened epithelium does not cover the rhopalial ridge but forms a simple band on each side until it reaches nearly to the mouth of the pocket, where the first pits appear. The pitted epithelium lines the angle of the pocket and fills one-half or two-thirds of it. The pockets are smaller and the pits less numerous than in the *Dactylometras* 18-25 mm. in diameter, described above, and, moreover, there is no extension of the pitted epithelium outward along the floor of the pocket, which is lined by the same kind of epithelium as the roof.

Dr. Brooks has figured a young *Dactylometra* which has its third set of tentacles just budding. This specimen is 70 mm. in diameter and is therefore considerably older than my specimens, which are probably in the first half of the *Chrysaora*-stage and my comparison of them with the condition in *Chrysaora* of about the same size is the proper one. It would appear, then, that in the *Chrysaora*-stage of *Dactylometra* the structures in the sensory niche are more advanced, while the dorsal groove is, if anything, less advanced than in the same stage in *Chrysaora*.

SUMMARY

1. The eight-armed ephyra of *Chrysaora* has between the lap-pets at the end of each arm a rhopalium not enclosed in a sensory niche. The rhopalium is covered by a columnar epithelium, overlying a layer of nerve fibers. Within it contains at its distal end a mass of otoliths enclosed in endodermal cells that are continuous with the columnar epithelium lining the rhopalial canal, which is continuous with the gastric pouch of the arm. From the sides of the gastric pouch an endodermal lamella extends through the mesogloea to the ectoderm.

2. In the *Pelagia* stage of *Chrysaora*, 6 mm. in diameter, the rhopalium is enclosed in a sensory niche, covered by a hood, and is attached to the underside of the hood. From the point of attachment a rhopalial ridge runs centrally to the proximal wall of the niche. On each side of the rhopalial ridge there is an invaginated fold of epithelium consisting of small, ciliated, cuboidal cells and covering a layer of nerve fibers that is continuous with the layer of nerve fibers upon the rhopalium. This epithelium contains shallow secondary pits, and at some point beneath it the layer of nerve fibers comes into contact with the endodermal lamella.

3. At the beginning of the *Chrysaora*-stage of *Chrysaora* when the animal has reached a diameter of 10 mm. the dorsal sensory groove makes its appearance as a shallow depression in the exumbrella over the base of the rhopalium, but is still lined by flat epithelium like that of the general surface.

4. In the adult *Chrysaora* the dorsal sensory groove has become a deep conical depression and is lined by columnar, ciliated epithelium with an underlying stratum of nerve fibers. The topography of the sensory niche is heightened and the cuboidal pitted epithelium of the sides of the rhopalial ridge extends on each side into a deep lateral pocket that projects centrally beyond the opening of the rhopalial canal so that its fundus occupies a ridge on the roof of the gastric pouch. In this area the layer of nerve fibers comes into close contact with the endodermal epithelium as it goes along the whole extent of the rhopalial canal. These pockets

are probably similar to structures found by Eimer and Claus in *Cyanea* and *Aurelia*.

5. The adult *Pelagia cyanella* possesses a shallow dorsal sensory groove. The rhopalium is similar in all important respects to that of *Chrysaora* and is attached to a short and low rhopalial ridge which is covered by a columnar epithelium overlying a layer of nerve fibers, among which are large bipolar ganglion cells. In the nerve-fiber layer of the rhopalium are found nuclei probably belonging to small ganglion cells. There is no pitted epithelium at the sides of the rhopalial canal nor lateral pockets, and there is no thickening of the epithelium of the niche. The endodermal lamella may be traced in contact with the ectoderm of the side of the rhopalial ridge to the base of the rhopalium.

6. A comparison of the *Pelagia*-stage of *Chrysaora* with the adult *Pelagia* shows that both possess in each of the eight principal radii a rhopalium lying in a well developed sensory niche, which is lined for the most part by undifferentiated epithelium. Both have a thickening of the epithelium on the surface of the rhopalial ridge. They differ in that *Pelagia* has no pitted epithelium while in this stage *Chrysaora* has not yet acquired a dorsal sensory groove. If Hesse be right in saying that *Pelagia* likewise has no true dorsal sensory groove, the difference between the two forms becomes just so much less. But if it be not sensory, it is difficult to understand the presence of this persistent dimple in two species of *Pelagia*.

7. The adult *Dactylometra* presents in its sensory apparatus all the characters of *Chrysaora* in an exaggerated degree. The pitted epithelium covers a greater surface and is more highly developed. It fills the whole of the lateral pockets with closely packed branching tubules.

8. Of the larval forms of *Dactylometra*, the ephyra is essentially like the same stage in *Chrysaora*. The *Pelagia* stage has not been studied. A larva in the *Chrysaora*-stage, 18 mm. broad with the secondary tentacles 3 mm. long, shows the rudiment of a dorsal sensory groove consisting of a flat disc of columnar epithelium. The rhopalium is like that of the adult *Chrysaora*. But the rhopalial ridge is very short and completely covered by the

pitted epithelium which extends centrally into very large lateral pockets, where it covers only the floor of the pocket. In the distribution of the pitted epithelium and the form of the lateral pockets the *Chrysaora*-stage of *Dactylometra* differs from *Chrysaora* of the same size.

9. Sections of the rhopalia of all of these forms fail to afford any evidence for Hesse's theory of the intercellular origin of the otoliths. On the contrary, they appear to be formed within the cells, instead of between them. It is true, as Hesse says, that there is an unbroken gradation between the otolith cells and the columnar, endodermal epithelium of the rhopalial canal. The same gradation is found between the chorda-cells and the columnar epithelium at the base of the solid tentacles from which the rhopalia are developed in the scyphistoma (see Bigelow, '00, pl. 37, figs. 53-56), but it is not evident what bearing this fact has upon the probable origin of the otoliths.

10. The *Pelagidae* show no layer of nerve fibers underlying the endodermal epithelium and nerve fibers of the dorsal sensory groove do not appear to penetrate the mesogloea. In this region the mesogloea of *Dactylometra* appears to be quite structureless, although fibers are abundant in it elsewhere. There may be a nervous communication between the nerve centers in the sensory niche and the endoderm through the endodermal lamella, but if present its demonstration will require special methods of staining, which were not employed in the present investigation.

11. In considering these results due allowance must be made, of course, for individual variation and for liability to error due to shrinkage and distortion of specimens. There is also a possibility of confusing larval forms when they are not reared from the egg. Still I think that in all essential particulars the results given above will be confirmed by future investigations.

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EXPLANATION OF FIGURES

All figures are camera drawings, except as otherwise noted below. They were made at the magnification indicated and reduced in reproduction to about one-third the original size.

LETTERING COMMON TO ALL THE FIGURES

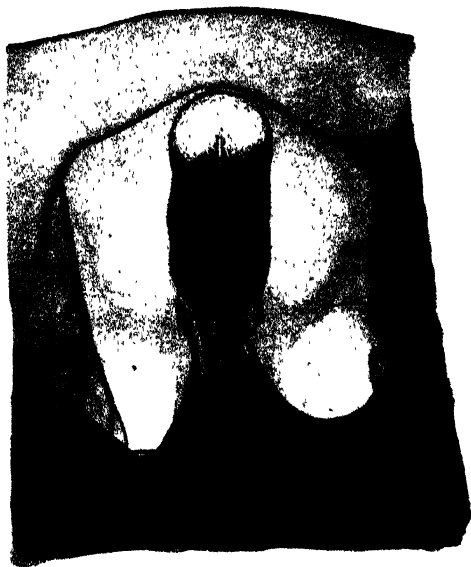
<i>b.</i>	Battery of nettle cells.	<i>p.</i>	Lateral pocket lined with pitted epithelium.
<i>c.</i>	Concretions, or otoliths.	<i>p.e.</i>	Pitted epithelium.
<i>ec.</i>	Ectoderm.	<i>r.</i>	Rhopalium.
<i>en.</i>	Endoderm.	<i>r.c.</i>	Rhopalial canal.
<i>g.</i>	Ganglion cell.	<i>r.r.</i>	Rhopalial ridge.
<i>h.</i>	Hood.	<i>s.e.</i>	Sensory epithelium.
<i>m.</i>	Muscle fibers.	<i>s.g.</i>	Dorsal sensory groove.
<i>m.l.</i>	Marginal lappet.	<i>s.n.</i>	Sensory niche.
<i>m.p.</i>	Marginal endodermal pocket.		
<i>n.</i>	Layer of nerve fibers.		

Figs. 1-12, 21-34, *Chrysaora*; 13-16, *Pelagia*; 17-20 and 35-38, *Dactylometra*.

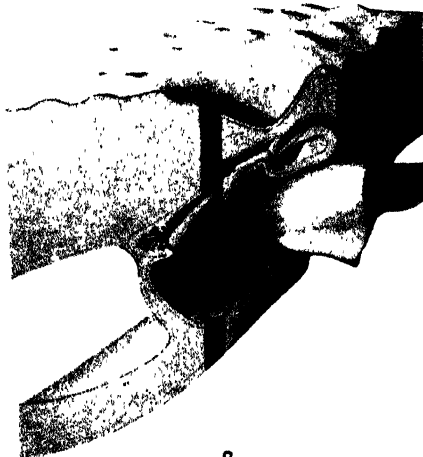
1 View of the sensory niche of a *Chrysaora* as seen from the under side by transmitted light. The specimen from which this was taken was a young one 25 mm. in diameter with 24 tentacles. $\times 200$.

2 Portion of the margin of the umbrella of a full grown *Chrysaora* containing the rhopalium and adjacent organs. The cut surfaces are in tangential and radial planes. $\times 56$. This figure is a reconstruction from sections.

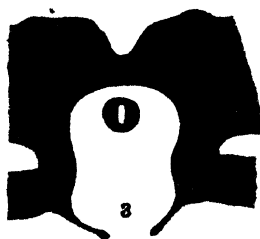
3-9 Diagrams from typical sections of the series used in the construction of fig. 2. $\times 56$. Fig. 3 corresponds to plane I in fig. 10, fig. 4 to II, fig. 5 to III, fig. 6 to IV, fig. 7 to VI, fig. 8 to VII, and fig. 9 to VIII.



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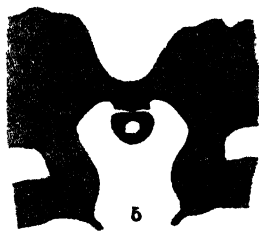
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EXPLANATION OF FIGURES

10 Diagrammatic drawing of a radial section of the margin of *Chrysaora*, cutting the rhopalium longitudinally. $\times 56$. The lines I, II, etc., show the planes of figs. 3, 4, etc.

11 A vertical tangential section cutting the rhopalium obliquely so as to show the band of nerve fibers running from the rhopalium to the lateral pocket. Slightly diagrammatic. $\times 120$.

12 Radial section through the columnar epithelium at the proximal end of the rhopalial ridge. This section happens to contain two nettle cells. $\times 550$.

13-15 Typical tangential sections through the sensory apparatus of *Pelagia cyanella*, somewhat diagrammatic. $\times 108$. Fig. 13 corresponds to fig. 4 of *Chrysaora*, fig. 14 to a plane between figs. 5 and 6, and fig. 15 approximately to fig. 8.

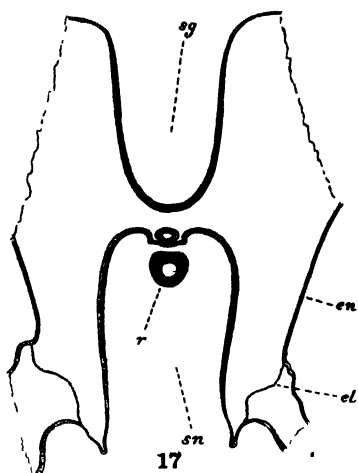
16 A radial section of the rhopalial ridge at its proximal end in *Pelagia*, showing a large ganglion cell. $\times 1120$.

EXPLANATION OF FIGURES

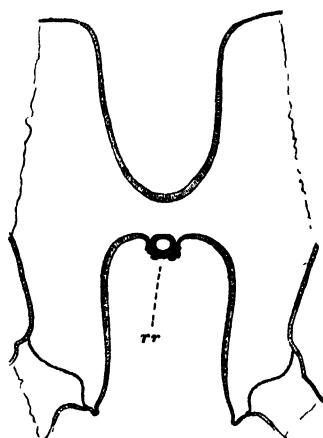
17-20 A series of sections from *Dactylometra quinquecirrha*. $\times 56$. Fig. 17 corresponds to fig. 5 of *Chrysaora*, fig. 18 is in a plane corresponding to V of fig. 10, fig. 19 corresponds to fig. 7, and fig. 20, which is oblique to the radius, nearly corresponds to fig. 8.

21 The ends of one of the arms of an ephyra of *Chrysaora* viewed from the under side as a transparent object, showing the relations between the rhopalium, the gastric pouch (*r.c.*), and marginal lappets. $\times 280$.

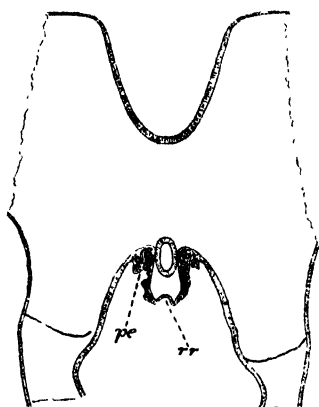
22 Longitudinal section of a rhopalium from the same specimen. $\times 600$.



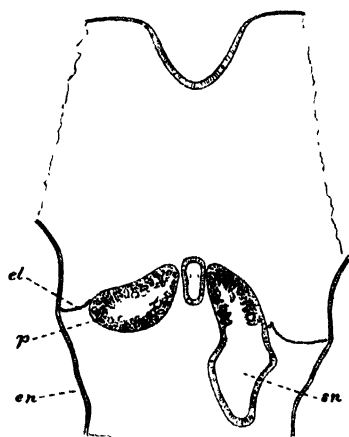
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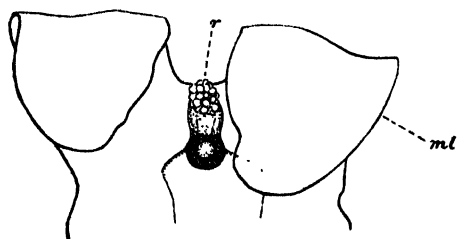
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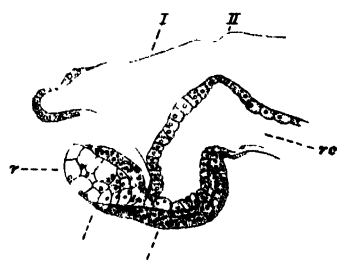
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EXPLANATION OF FIGURES

23-25 Tangential sections of another arm of the same specimen. $\times 195$. Fig. 23 is about in plane I of fig. 22, fig. 24 is in plane II, while fig. 25 is a section from near the base of the arm.

26 Radial section through the medial line of the rhopalium of a *Chrysaora* larva that is in the *Pelagia* stage and is 6 mm. in diameter. $\times 490$.

27 A section of the same specimen parallel to fig. 26, just to one side of the rhopalium, showing a cross section of the rudimentary lateral pocket. $\times 490$.

28-31 Tangential sections across another rhopalium of the same individual. $\times 490$. Fig. 28 is in plane I, fig. 29 is in plane II, fig. 30 is III, and fig. 31 in IV of fig. 26.

32 A tangential section oblique to the axis of the rhopalium and through the longest diameter of the rudimentary lateral pocket. $\times 490$.

EXPLANATION OF FIGURES

33 Radial section through the rhopalium of a young *Chrysaora* just passing into the adult form. The specimen was about 10 mm. broad and the second set of tentacles about as long as the marginal lobes. $\times 300$.

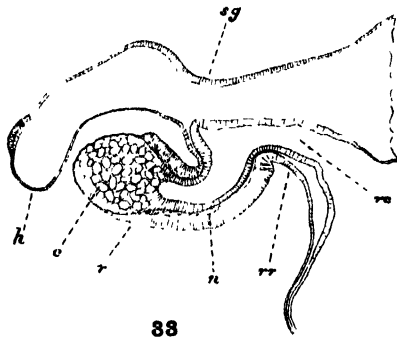
34 A section at right angles to the above through another rhopalium of the same animal. $\times 280$.

35 A radial section through the rhopalium of an ephyra of *Dactylometra*. there has evidently been considerable shrinkage of the mesogloea in this specimen. $\times 523$.

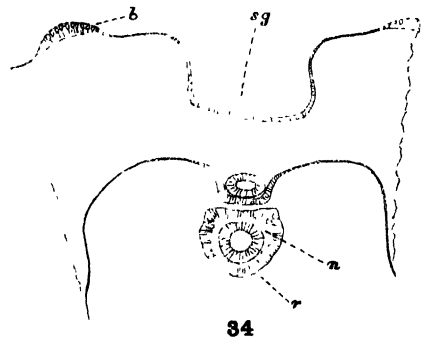
36 A similar section from a somewhat older larva. $\times 490$.

37 A tangential section through the sensory niche just proximal to the base of the rhopalium in a young *Dactylometra* about 18 mm. in diameter, which is in the early part of the *Chrysaora* stage. $\times 190$. (Compare figs. 7 and 18).

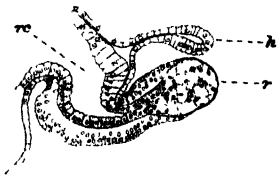
38 A section parallel to the above about 60μ farther inward through the outer half of the lateral pockets. $\times 190$.



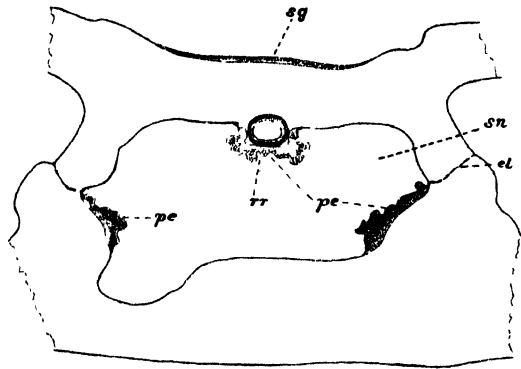
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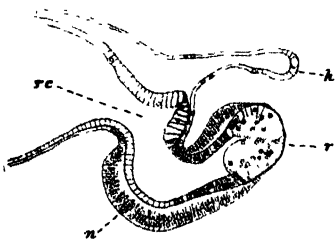
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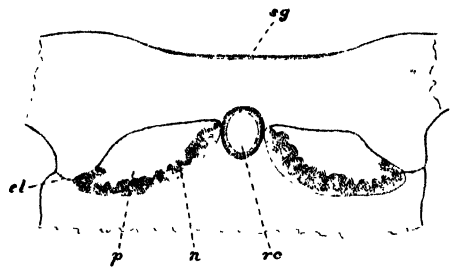
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THE OUTGROWTH OF THE NERVE FIBER AS A MODE OF PROTOPLASMIC MOVEMENT

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THIRTY-TWO FIGURES

THREE PLATES

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INTRODUCTION

The idea that protoplasmic movement is concerned in the activities of the nervous system has appeared in a variety of forms during the past twenty years. Not only has it been supposed that the processes of nerve cells may be extended and withdrawn, making and breaking connections with other cells during functional activity, but also that the movement of cells and their processes in the course of development has been the chief factor in bringing about the specific nervous connections found in the adult.¹ The latter idea is associated particularly with the name of Ramon y Cajal, who in his memoir on the retina ('92) first put

¹ Schiefferdecker ('06) has discussed at length and in an admirable way the extensive literature bearing upon this subject.

forth the hypothesis of chemotaxis to account for these supposed movements. The discovery, by the same observer ('90), of the *cônes d'accroissement*, found at the end of embryonic nerve fibers very early in their development, had given a clue as to what this growth mechanism might be, for the resemblance of the minute processes borne upon the terminal enlargement of the growing nerve to pseudopodia, naturally suggested that this structure might owe its peculiarities to amoeboid activity. In his larger work on the structure of the nervous system Cajal ('99) elaborates his theory more fully and leaves no doubt as to his meaning regarding the activity of the growth cones. After describing their appearance he says (p. 544-5): "From the functional point of view, the cone of growth may be regarded as a sort of club or battering ram, endowed with exquisite chemical sensitiveness, with rapid amoeboid movements, and with a certain impulsive force, thanks to which it is able to press forward and overcome obstacles met in its way, forcing cellular interstices until it arrives at its destination." From this it is seen that Ramon y Cajal took a considerable step in advance of His ('86-'90), and placed upon a still firmer basis the concept that the nerve fiber is formed as the outgrowth of a single cell.

Although this view has enjoyed wide acceptance, the opposing theory of Hensen ('64-'08), which denies that there is a free outgrowth of protoplasmic substance to form the nerve fibers, has met with increasing support within the past few years, especially in the work of O. Schultze ('04-'08), Braus ('04-'05), Held ('06-'09), Paton ('07) and Schaeppi ('09); and it seems that we are really very far from a satisfactory solution of the question, which even the invention of new and marvelously refined histological methods has failed to bring to a final settlement. Nor has Held's² compromise theory, which is based upon such methods, and which sees in Hensen's protoplasmic bridges merely a sort of substratum into which the fibrillar substance extends from the neuroblasts or ganglion cells, succeeded in har-

² Held's view appears on the surface to be a modification of Hensen's theory and it is usually classed as such, but a full examination of his complete work shows that in reality it approaches much more closely to His's view.

monizing the two views. The wide discussion of the subject which has taken place reached a certain culmination in the controversy between Held and Ramon y Cajal in the years 1906-1909, in which it became clear that the evidence for and against the two theories respectively, rested upon such minute histological details that a decision to which all would subscribe was impossible of attainment. These two observers studied to a great extent similar material, often by the same methods, and, in fact, their prepared material was so much alike that Ramon y Cajal, after seeing Held's specimens, expressed great astonishment at the similarity.³ Yet the respective interpretations given by them differ diametrically.

Under such conditions a search for evidence of other kinds is indicated. It was with the hope that a study of the problem by entirely different methods might yield such evidence, that the work described in the present paper was undertaken. A crucial experiment was sought that would decide between the two theories. That a decision of this question is of fundamental importance becomes apparent when we consider that the analysis of the factors bearing upon the development of this most intricate system of organs is wholly dependent upon it; for it is obviously impossible to study intelligently the mechanics of development of the nerve paths, unless we know whether we are dealing primarily with phenomena of protoplasmic movement or with mere progressive differentiation without movement.

An extensive series of experiments, as well as observations upon normal embryos, had led me previously to the adoption of the view of His and Ramon y Cajal. These experiments (Harrison '06-'10) showed that the ganglion cells within the nerve centers are the one essential element in the formation of the nerve fiber, inasmuch as pieces of the embryonic nervous system transplanted to any part of the body may give rise to nerve fibers, while no fibers ever develop in the absence of ganglion cells. It was recog-

³ R. y Cajal, 1908, p. 3, footnote: *Tout récemment pendant un voyage en Allemagne, nous avons eu le plaisir d'examiner à Leipsig, les excellentes préparations de M. Held. Ainsi que nous l'attendions elles sont très réussies, mais à notre grande surprise elles montrent à peu près les mêmes images que les nôtres.*

nized, however, that in all of the first experiments the nerve fibers had developed in surroundings composed of living organized tissues, and that the possibility of the latter contributing organized material to the nerve elements, stood in the way of rigorous proof of the view that the nerve fiber was entirely the product of the nerve center. The really crucial experiment remained to be performed, and that was to test the power of the nerve centers to form nerve fibers within some foreign medium, which could not by any possibility be suspected of contributing organized protoplasm to them.

Two lines of experimentation were taken up with this end in view. The one was to introduce small pieces of clotted blood into the embryo, in the path of the developing nerves. This gave positive results, in that nerve fibers were found several days after the operation, extending from the medullary cord into the blood clot, and the sole possible disturbing factor in these experiments was the presence of scattered embryonic cells, which began to organize the clot within two days after its transplantation (Harrison '10).

The second line of experimentation, which consisted in the isolation of pieces of living tissue in unorganized media, gave considerable difficulty at first, but in the spring of 1907 a method was finally devised, which satisfactorily accomplished the purpose.

The present paper contains a complete account of these experiments, which have been described previously in a preliminary notice.⁴ In addition, a brief description of the early development of the nerve elements in the normal amphibian embryo

⁴ The first of these experiments were made in the Anatomical Laboratory of the Johns Hopkins University. After my removal to Yale University they were continued during the seasons of 1908 and 1909 in the Sheffield Biological Laboratory. The repetition of the work gave results which not only confirmed those of the first season, but which also met many possible objections that might have been raised against the original experiments. The preparations obtained during the second season's work were, on the whole, much more convincing than those of the first, and they have been used almost exclusively in making the illustrations for the present paper. The first account of the work was given in a paper before the Society for Experimental Biology and Medicine in May 1907, and later the results were incorporated in a lecture before the Harvey Society of New York, in March 1908.

will be given here, in order to afford a basis for comparison with the protoplasmic filaments formed by the isolated pieces of nervous tissue. Fortunately the part descriptive of normal development need not occupy very much space, for we now have a large mass of facts available in the recent work of Ramon y Cajal ('07-'08) and in the exhaustive monograph of Held ('09).

The method which I have used is, in a word, as follows: Small pieces of embryonic tissue, taken before the histological differentiation of nerve fibers has begun, are placed in hanging drops of lymph, and the sealed preparations kept under observation for a number of days. It is found that the embryonic cells under these conditions manifest striking amoeboid activities, which are especially pronounced in cells taken from the nervous system, and result in such cases in the formation of long threads of hyaline protoplasm. These fibers bear a perfect morphological resemblance to undoubted nerve fibers found in sections of normal embryos of a corresponding stage of development. So striking is the similarity between these structures that no hesitancy is felt in regarding them as identical with one another.

This method, which obviously has many possibilities in the study of the growth and differentiation of tissues, has two very distinct advantages over the methods of investigation usually employed. It not only enables one to study the behavior of cells and tissues in an unorganized medium free from the influences that surround them in the body of the organism, but it also renders it possible to keep them under direct continuous observation, so that all such developmental processes as involve movement and change of form may be seen directly instead of having to be inferred from series of preserved specimens taken at different stages. While these two advantages have not heretofore been combined in a single mode of procedure, the first named has been attained by Loeb ('02) who has embedded pieces of tissue, chiefly epidermis, in blocks of agar or clotted blood and transplanted them to spaces in the body of living animals. It is interesting to note that under these conditions epithelial cells undergo changes which apparently resemble closely the activities of embryonic cells observed in the present investigation, as a comparison of Loeb's figures with my own shows.

EARLY DEVELOPMENT OF NERVE FIBERS IN THE NORMAL EMBRYO

Conditions obtaining in the central nervous system antecedent to the differentiation of fibers

In the walls of the medullary groove and the medullary tube just after it has been completely folded off from the epidermis, one can distinguish in sections a number of irregular layers of cells, mostly oval in shape, with long axis placed radially with respect to the tube as a whole. Sometimes these cells are seen to be bound by a membrane, but usually they are indistinctly defined except where they are deeply pigmented, in which case the pigment granules are thickest around the periphery of the cells. At this period the individual cells do not extend through the whole thickness of the tube from the central canal to the external limiting membrane.

In slightly later stages, *i.e.*, when the tail bud is barely distinguishable, the epithelial cells begin to stretch out radially and then many of the individual cells are seen to extend from the inner to the outer wall of the tube. The boundaries remain indistinct, unless, as before, the cells are marked off from their neighbors by pigmentation. After the elongation of the epithelial cells constituting the walls of the medullary tube has taken place, it is seen that certain cells, less elongated in form, and containing a round nucleus, remain in the outer zone of the wall of the tube. These are the first of the neuroblasts of His, the cells destined to give rise to the nerve fibers.

There are as yet no peripheral nerves, nor are there any nerve fibers visibly differentiated within the walls of the medullary tube. The cranial ganglia are marked off and occupy approximately their definitive position, and in the anterior part of the trunk region the ganglion crest is beginning to break up, its cells extending to the dorsal border of the muscle plates. In the middle of the trunk the crest is intact and it rests entirely upon the medullary cord, while near the tail bud it can scarcely be distinguished at all.

At this point it will be profitable to inquire a little more fully into the supposed syncytial nature of the central nervous system. When sections alone are studied, there may be an apparent justification for regarding the walls of the neural tube as a mass of protoplasm with nuclei embedded in it,⁵ for, as has already been pointed out, the cell boundaries within the medullary cord are difficult to make out unless they happen to be indicated by pigmentation. When examined in the fresh condition, an entirely different state of affairs is revealed. It is astonishing how easily the cells, which in sections seem to be baked together in a mass, come apart when the medullary cord is dissected out of the living embryo and teased in water or salt solution. The cells appear as round glistening vesicles under the binocular microscope, and under the oil immersion they are found to be very clearly defined, each being surrounded by a very delicate, though perfectly distinct, cell membrane. The cells are gorged with yolk granules, and the nucleus appears as a clear space near the center of each cell (fig. 15). There is not very much difference in the appearance of the cells in the different media named, though in water and the more dilute salt solution (0.2 per cent) there is some imbibition of water, which may result in the formation of a more or less eccentric clear zone just beneath the cell membrane (fig. 15 c). No sign of protoplasmic bridges can be made out. From these observations the conclusion seems clearly justified that the medullary cord of the frog embryo is made up of perfectly distinct cells. It is in no sense a syncytium, and statements to the contrary based upon the insufficient evidence from stained sections, are to be received with skepticism.

The medullary cord is sharply marked off from all surrounding structures except where the ganglion crest is breaking down. The cord is in direct contact with the muscle plates and the notochord, but in the angle between the two latter structures, and in the the grooves between successive somites there are small spaces, which at this period are entirely devoid of cells. Just what is the structure of the material that fills these spaces in the living

⁵ Cf. for instance Weyssse and Burgess (1906) on the histogenesis of the retina.

embryo is not, in my opinion, certain, but in sections of preserved specimens, as Held has described in great detail, a delicate network, is visible. The character of this intercellular reticulum varies from specimen to specimen and, as will be seen, varies very greatly according to the mode of preservation. It seems to be beyond doubt that the structures in question are due in part to coagulation, though just to what extent it is not easy to say. In order to test the matter a series of embryos were preserved in osmic acid, which, as Fischer ('01) has shown, fixes protoplasm without bringing about any visible change in structure, and which after prolonged action (24 hours, 1 per cent, in the case of *Amoeba proteus*) so fixes it that alcohol causes no further change. Sections of these embryos show plainly that the spaces between the organs described above are almost perfectly clear; only occasionally do very delicate filaments appear bridging the spaces. The contrast with specimens which have been preserved in a corrosive sublimate-acetic mixture is very great; and very much more pronounced still is the difference shown by embryos preserved in Hermann's fluid, which is, however, otherwise a very ill adapted preservative for this material.⁶ It is not intended on the basis of the foregoing observations to deny the existence of protoplasmic bridges in embryos of this stage, but it does seem proper to call attention to the facts just stated, in order to show the necessity for caution in ascribing significance to the connection between such fine structures and the developing nerve fibers.

Differentiation of nerve fibers

The embryo last described is in the stage which was used for most of the experiments. It is the oldest stage of which it can be said with certainty, without microscopic examination, that there are no nerve fibers present. In the next stage to be con-

⁶ On account of the large amount of yolk, which becomes very brittle after prolonged treatment with osmic acid, the amphibian embryo is not a favorable object for the study of this question. It was found necessary to impregnate the embryos with celloidin before embedding in paraffin, and even then the sections were not perfectly satisfactory. It would be of great interest to have an exact compari-

sidered, an embryo of *R. sylvatica*, 4.1 mm. long, the beginnings of the peripheral nerves, and of some of the principal central bundles are plainly visible. Of all the peripheral nerves the *r. ophthalmicus* of the trigeminal, seems to be furthest advanced. A very early phase of this nerve is shown in fig. 2 (*nf*), drawn from an embryo of *R. esculenta*, 3 mm. long, which is in about the same stage of development as the *sylvatica* embryo just mentioned. Protoplasmic processes of the cells within the ganglion are seen to extend for a short distance into the mesenchyme, without having any special relation to the cells of that tissue. The ends of the processes are branched and filamentous. In the *sylvatica* embryo under consideration, a considerable number of peripheral nerves in addition to the ophthalmic are already laid down. There are at least four ventral spinal roots, corresponding to the second, third, fourth and fifth muscle plates, to which they may be traced; several of the dorsal nerves of Rohon-Beard, extending out between the myotomes and the epidermis; and some fibers in the *r. lateralis vagi*.⁷

The early characteristics of the developing nerve are most clearly shown by the fibers which originate in the dorsal cells of Rohon-Beard. These grow just beneath the epidermis in the space between the muscle plates, where at this period there are no loose mesenchyme cells, and they remain free from sheath cells throughout their growth. The clearest cases of the earliest beginning of these nerves have been found in an embryo of *Rana palustris*, 3.6 mm. long, which is almost identical in degree of development with the *sylvatica* embryo just described. The

son of the protoplasmic bridges fixed in osmic acid with those seen after fixation in the usual preservatives made upon such vertebrate embryos as those of the selachian, the teleost, or the bird, in which there is little or no yolk in the tissues at the time when the first nerve fibers differentiate.

⁷ These early nervous connections, which are important for the proper interpretation of the relation between structure and function in the neuro-muscular system, have been ignored by a number of investigators. In his histogenetic study of the nervous system O. Schultze ('05) has overlooked these stages of development completely and has thereby been entirely misled in his views regarding the early development of nerve fibers and the formation of the cutaneous plexuses (Cf. Harrison '04, '06). Held ('09) has recently subjected Schultze's work to a searching criticism, all the main points of which seem to be entirely justified.

cells which give rise to the dorsal nerves form a column in the dorso-lateral part of the wall of the medullary tube just within the external limiting membrane. In this stage certain of the cells are seen to have put forth fine branched processes, which extend for a short distance laterally in the notch between successive muscle plates (fig. 1, *nf*). The processes end in extremely fine filaments, so fine that their exact delimitation is often very difficult to determine. The cell shown in the figure gives off another process quite as extensive as the one shown, but which is seen only in the section next to the one drawn. The structures in question are segmentally arranged, and correspond in the embryo under consideration to the intervals between the muscle plates from the second to the thirteenth segments. A much more advanced condition is shown in an embryo but very slightly older (3.7 mm. long). The dorsal nerves are here composed of several fibers in a bundle, each fiber connecting with a cell. The nerve shown in fig. 3 is composed of four such fibers (*nf*) which arise in pear-shaped cells (*nbl*) and converge toward the point where they leave the medullary cord between the second and third myotomes. The endings are not shown in the section because the fibers bend just beneath the epidermis and run dorso-ventrally. They stain intensely with Congo red, as do the cone-shaped processes of the cells from which they originate, and they show a fairly distinct fibrillation, even when stained merely by this method.

The ends of the fibers are best seen in sagittal sections taken just between the epidermis and the underlying muscle plates. In a series of sections made from an embryo of *Rana pipiens*, 4 mm. long, they show particularly well. In fig. 4 the end of a bundle of three fibers situated between the ninth and tenth segments is shown. This terminal structure (*npl*) consists of a mass of hyaline protoplasm having a form suggestive of a rhizopod. The mass extends out into a number of very fine filaments. Such structures are found in each segment. Another, more highly magnified, is shown in fig. 5. Further towards the head of the embryo the fibers are longer and more branched (fig. 6), each branch ending in one of the peculiar enlargements just described. The young fibers of the *r. ophthalmicus* end similarly, although the ending

cannot always be made out with such clearness, owing to the existence of the branched mesenchymecells in their immediate vicinity. In other nerves, as in the case of the *r. lateralis vagi* of *Amblystoma*, there is a slight enlargement at the end of the growing fiber, though branched filaments are not clearly shown there.

It is a striking fact that in these early stages of development, each nerve fiber, in fact each branch of a nerve fiber, ends in an enlargement of this kind. The enlarged ends, as well as the fibers throughout their whole length, are attached to surrounding organs by fine threads, but, as stated previously, I am unable to find any safe criterion to distinguish between natural protoplasmic filaments and products of coagulation. Aside from these fine filaments, the nerve fibers are found to end free, and anastomoses between different nerves are not present at this stage. This is perfectly clear in the case of the cutaneous nerves formed by the cells of Rohon-Beard. A little later, however, as seen in a *R. pipiens* embryo, 6 mm. long, the branches of the individual segmental nerves are found to have extended so far as to come into contact with those of the next segment, the result being the formation of a beautiful plexus of nerve fibers beneath the skin overlying the muscle plates. This is composed of fibers devoid of sheath cells, and in specimens hardened and stained by vom Rath's picro-platino-osmo-acetic mixture, the fibrillae are shown very clearly. Plexus formation is thus seen to be secondary, resulting from the accidental coming together of the growing ends of nerve fibers which have origin in different segmental nerves (text fig. 1). In all cases the nerve fibers are found to extend gradually out from the center, and the end of each small twig is characterized by an enlargement made up of hyaline protoplasm, provided with fine filaments, just as the main stem of the fiber itself is at first.

The above observations upon the ends of the developing nerves agree substantially with those of Ramon y Cajal, although they are based upon specimens preserved by entirely different methods. The enlarged ending provided with protoplasmic filaments is in all probability the *cône d'accroissement* first described by him, the filaments being shown in these cases perhaps more completely

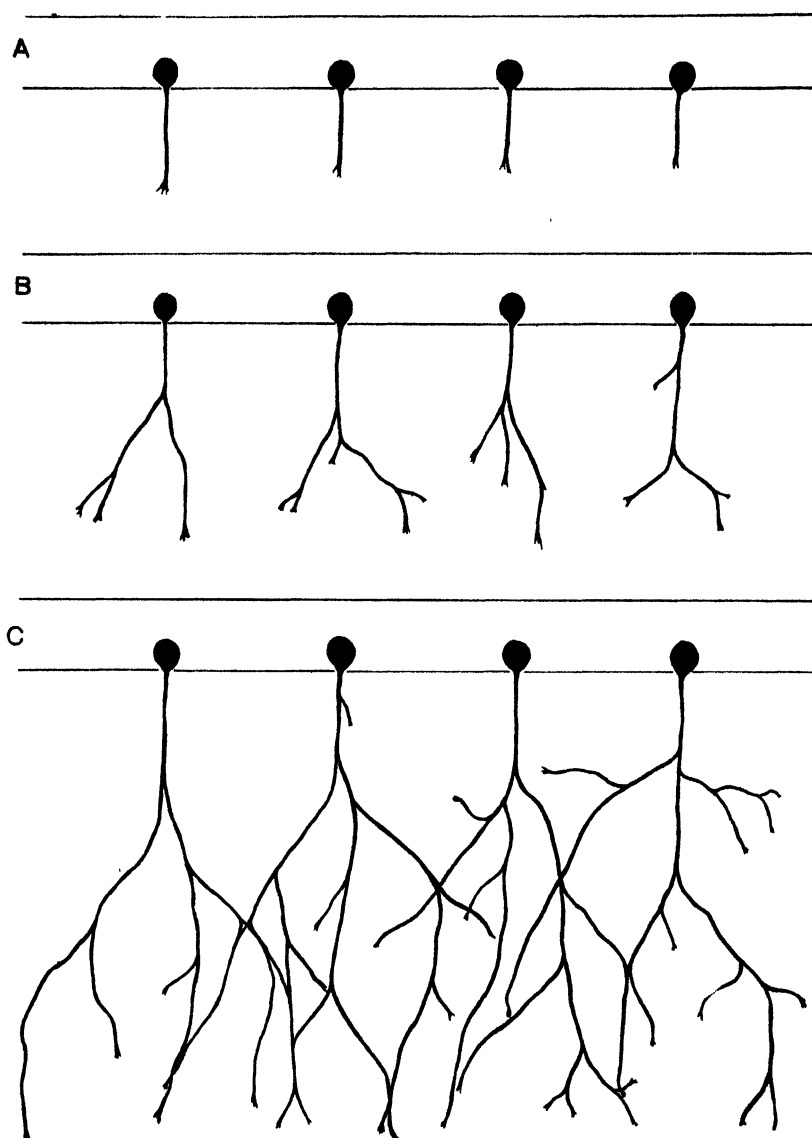


Fig. 1 Diagram illustrating the mode of development of the sensory nerve plexus, derived from the dorsal cells of Rohon-Beard. Each segmental nerve is represented in its simplest terms as a single fiber originating in a single cell of the neural tube. A, early stage in which the nerve fiber has just begun to grow out. B, Later stage in which each segmental nerve has begun to branch. C, Final stage in which neighboring nerves form anastomoses with each other.

by the ordinary embryological methods.* Cajal has figured in a number of places his growth cones, as seen both in Golgi and in silver nitrate preparations. Those shown in his book on the Structure of the Nervous System, vol. 1, p. 515, are in most striking agreement with the figures here presented. Again, there is no sharp discrepancy between these figures and those of Held, whose figures, like those of Cajal are sharper than the present ones, since they represent the specific coloration of the neurofibrillae. The only essential difference shown by those of the former observer is in the relation of the young nerve fibers to the protoplasmic net-work between the cells and this to my mind is wholly a question of interpretation. Considering the uncertain nature of the intercellular net-work, as pointed out above, the unusually positive views of Held regarding its rôle in the development of the nerve fibers seem but very insecurely founded.

EXPERIMENTS UPON EMBRYONIC TISSUES ISOLATED IN CLOTTED LYMPH

Description of methods

The first attempt which I made to study the development of isolated bits of embryonic nervous tissue gave entirely negative results. The tissue was dissected out from the embryo and put either into physiological salt or Locke's solution, but no differentiation was observed, before disintegration began. Later a more natural environment for the isolated tissue was sought in the ventricles of the brain and in the pharynx of young embryos. The tissues were transplanted to these cavities and the specimens were killed after from two to seven days and examined in serial sec-

* In salmon embryos preserved and stained by the ordinary embryological methods, no protoplasmic filaments are shown attached to the growing ends of the nerve fibers within the central nervous system, and for this reason the latter were figured as smooth in my paper on the histogenesis of nerves (Harrison '01). Ramon y Cajal has pointed out that this condition is likely due to the insufficiency of the methods. While I agree that there is some ground for this criticism, it seems nevertheless probable that there are actual differences between the growing ends found in different places and in different species.

*
tions. These experiments likewise resulted negatively. In no case were nerve fibers found extending from the transplanted piece free into the cavity, although the pieces themselves often showed differentiation of fibers, and in cases where the graft had grown fast to the walls of the medullary tube, fibers passed from the former to the latter. The only conclusions which could be drawn from these results were either that the nerve fibers were built up by the differentiation of formed protoplasmic structures, according to the view of Hensen and Held, or else that the growing fibers were positively stereotropic and hence remained within the solid tissue instead of passing out into the surrounding fluid.

Acting upon the latter assumption, the next step was to try a fixed medium. Two such were employed, one of which, gelatine, gave no results at all, the transplanted embryonic tissue remaining entirely unchanged after imbedding. The other, clotted frog's lymph, gave the results that are here recorded. It was rather to be expected that this medium would yield positive results, if indeed such were to be obtained at all, for it would be chemically the most natural medium, and the fine net-work of fibrin threads, bathed by the fluid serum, would in a measure simulate mechanically the protoplasmic net-work, which, according to Hensen, Held and others, seems to exist in the tissue spaces in which the peripheral nerves undergo their early development.

In the first experiments made with the lymph, the technique employed was comparatively simple. The tissue to be studied was dissected out of the embryo under the binocular microscope in 0.4 per cent sodium chloride or in Locke's solution without sugar. It was then transferred to a cover-slip by means of a capillary pipette, and a drop of lymph drawn from one of the lymph sacs of an anaesthetized frog was quickly dropped upon it. The cover-slip was then inverted over a depression slide and the preparation kept in a moist chamber. In order to avoid evaporation while the specimens were under examination it was necessary to seal the preparations, which was done most satisfactorily by applying melted paraffine around the cover-slip with the edge of a warm plate.

Although the first definite results were obtained by the above methods, it was found that bacteria quickly invaded the preparations, often destroying them as soon as the second day after implantation. Continued observation over a long period was therefore impossible, and many otherwise good specimens were spoiled before they had yielded anything but negative results. After experimenting a little with antiseptics such as thymol and acetone-chloroform, it became apparent that satisfactory preparations could not be obtained except by working aseptically. The procedure necessary for this involved much tedious detail, though it offered no insuperable difficulties.⁹ All glassware, such as slides, covers, pipettes and dishes, was sterilized by dry heat, either in a hot air sterilizer or by passing them through a flame. For cloths and filter paper an Arnold sterilizer or an autoclave was used, and the needles, scissors and forceps were sterilized by boiling. The sterilization of the embryos and the frogs from which the lymph was to be taken offered greater difficulties, and in fact was accomplished only approximately, though the number of organisms seems to have been so reduced as not to interfere with the purpose of the experiments. The embryos were simply cut out of their jelly in water which had been boiled or passed through a Pasteur-Chamberland filter. They were then washed in about six successive changes of this water. The salt solution in which the operations were performed was sterilized in the same way. The frogs were chloroformed and then washed thoroughly in sterile water, laid out upon moist filter paper and kept in a covered dish. In some cases they were first washed in mercuric chloride (0.1 per cent) and in others they were kept for 24 hours before chloroforming in a solution of copper sulphate one part to 500,000, but I am not prepared to say whether these means were sufficiently effective to be of material advantage.

The results of these manipulations are altogether satisfactory as regards asepsis, although the making ready of the apparatus consumes so much time, and the constant attention to the

⁹ I am greatly indebted to Prof. Leo F. Rettger for valuable suggestions as to this procedure, and for his generosity in putting at my disposal the apparatus in his laboratory.

details of manipulation during operations is so fatiguing, that only a small number of preparations can be made in one day. Many preparations proved to be absolutely sterile. In some of these the tissues were kept alive for over five weeks, and in a great many for one or two weeks. Some were contaminated, most frequently with *Bacillus subtilis*, but even in these cases the organisms did not usually appear in sufficient number to injure the living tissue until after it had been kept under observation for four or five days, which was long enough for the present purpose. Several epidemics of mould (*Penicillium*) were encountered, but this too grew slowly, usually from a single spore or two, and as it does not seem to kill the embryonic cells, it interfered but little with observations.

The tissue to be studied is dissected out from the sterilized embryos in a small flat dish containing dilute salt solution. After this is done the next step is to obtain a drop of lymph from the frog, which has already been prepared. The animal is suspended or placed in an upright position, and after cutting into the lymph sac near its upper end a long fine pipette is introduced and a small drop is drawn from the bottom of the sac. This is then placed upon the cover-slip, and the piece of tissue is quickly transferred to the lymph by means of the same pipette, with care to take with it as little of the salt solution as possible. Then the cover-slip is inverted over a depression slide and the preparation sealed by means of paraffine. It is important to have the depression in the slide deep enough to prevent the drop, which must also be small, from coming into contact with the bottom.

The procuring of the lymph is the most difficult part of the whole procedure, and the variability in its quality and in the amount obtainable, introduces into the work an element of inconstancy, which is a serious disturbing factor, preventing, as it does, a sharp clear-cut process of experimentation with exact controls from being carried out. The composition of the lymph varies not only amongst individual frogs but also in the different lymph sacs of the same individual, according to the position in which the animal has lain, the time since anaesthetization, and other factors of unknown nature. In general it may

be said that the first lymph drawn is the best; it clots readily and is less hemorrhagic, though this is by no means always the case. Usually a single frog can be used for five or six drops. The first two drops were taken in most cases from the femoral sacs. After opening these sacs, the lymph in the crural sacs becomes so watery that it will not form a sufficiently firm clot for the purpose, but the abdominal, lateral, and dorsal sacs of the trunk, as well as those of the forelimb will usually each yield a small drop which clots firmly. The quantity obtainable from a single sac is often too small to be of use. In fact, whenever any very large amount is to be had, it is very watery in quality, as is especially the case in the sacs which happen to lie lowermost. This oedematous condition is no doubt due to weakening of the heart, action but oddly enough it is more pronounced in frogs which have been pithed than in those chloroformed. Perhaps if the animals were anaesthetized by cold, the lymph obtained would be more uniform, and the low temperature would retard the clotting somewhat, which would be a distinct advantage. Even after taking the foregoing circumstances into consideration it is impossible always to get lymph of the proper composition. It may be very thin and fail to clot; or it may be so rich in fibrinogen that it clots immediately, even before it can be got out of the pipette, or in any case before the tissue can be transferred to it upon the cover. During this time, which is variable, some evaporation takes place and thus another factor of uncertainty is introduced. Still another variable is the amount of lymph relative to the amount of salt solution taken up with the embryonic tissue. It is not surprising, therefore, that there should be variations in the results of the experiments, which cannot be ascribed to any particular cause. On the other hand it apparently makes no difference from what species of frog lymph is taken, *Rana sylvatica*, *pipiens*, *palustris* and *clamitans*, all having yielded satisfactory material. Nor does it seem to be of consequence that the lymph should be of the same species as the embryonic tissue.

Embryos of *R. sylvatica*, *R. pipiens*, *R. palustris*, and, in a few experiments, of *Bufo lentiginosus*, were used, all in very nearly the same stage of development, corresponding to that used in most

of the previous experiments upon the development of the nervous system. The medullary folds are just completely closed and the tail bud is barely visible. The reason for choosing this stage is because it is the latest in which there is no histological differentiation in the nervous system or muscle plates. All of the cells are compact and no fibers whatever are present. The tissues are thus got into the lymph before their histogenetic development has begun.

The transference of the tissue to the lymph drop cannot be accomplished without a considerable amount of tearing. Often single cells or small groups are torn loose from the main mass and individual cells are fragmented, setting free yolk and pigment granules, but the fibrin holds the main masses together, unless the lymph is too thin, in which case the embryonic cells round off and separate from one another. This same kind of disintegration has been observed also in some cases in which the clot was firm. Even in the absence of bacteria the cells in these specimens may remain entirely unchanged, manifesting none of the peculiar protoplasmic activities seen in successful preparations. It has not been possible to assign any particular cause for this condition, and it must be attributed to slight deviations from the normal in the composition of the medium. All such experiments, and these have formed but a small percentage of the whole, have been rejected as inconclusive, and have been so indicated in the tabulation of results.

While the methods of preparation were practically the same in all cases, the experiments themselves were varied considerably as regards the tissues isolated. The chief object of the work being to test the power of embryonic nerve cells to form fibers by outgrowth, the largest number of experiments were made with nervous tissue. In some cases the medullary cord was dissected out entire, though it usually broke when transferred, and in others it was purposely fragmented by teasing. In quite a number of cases portions of the muscle plates were left attached to the medullary cord. In other experiments pieces of ectoderm from the branchial region, together with the underlying ganglia were taken. The behavior of this tissue, as regards the formation of fibers, was altogether similar to that of the medullary tube, and has

thus served to confirm the conclusions which have been drawn from the study of the former. On the other hand, the experiments have been controlled by observing the behavior of other embryonic tissues, such as muscle plates, ectoderm from the abdominal region, notochord, and yolk endoderm, under the same conditions. The results have shown, that while all tissues have certain features in common, each has nevertheless its specific activities, and these peculiarities coincide, as far as they go, with the activities shown by the respective tissues in the normal embryo. In other experiments separate pieces of ectoderm or muscle plates were placed in the lymph close to the nervous tissue, with a view to testing the power of the former tissues to influence the growth of nerve fibers. For instance in some experiments the medullary cord of the trunk was divided into its dorsal and ventral portions, and each was implanted separately with pieces of epidermis or of myotome, in the hope that it might be possible to show in this way that each of these tissues exerted some characteristic influence upon particular kinds of nerve fibers, the epidermis upon the sensory and the muscle tissue upon the motor. The results of the latter experiments were entirely negative; but since they were few in number and since the conditions of experimentation were not ideal, hope that this method may ultimately yield important discoveries need not necessarily be abandoned.

The total number of preparations made was 211. Permanent records have been kept for 150 of these, the remaining ones having given no promise from the beginning. Of the 150 cases, 35 have been rejected because they were found to be in bad condition before they could be expected to yield positive results. Table 1 shows how the experiments were distributed amongst the various embryonic tissues.

The specimens were studied almost exclusively under the water immersion lens, D* of Zeiss. In fact this lens is almost indispensable for the work. It has such a long working distance that the depths of the preparation can be readily examined without fear of breaking the cover. The magnification obtainable by the combination of this objective with eye-piece No. 4 is about 400 diameters, which is sufficient for all practical purposes. It was

TABLE 1

Summarizing the results of the experiments

TISSUE ISOLATED	NUMBER OF CASES RECORDED	NUMBER OF CASES REJECTED AS BELOW STANDARD	NUMBER OF VALID CASES	NUMBER OF CASES IN WHICH PROTOPLASMIC NERVE FILAMENTS WERE FORMED	PROPORTION OF CASES SHOWING NERVE FILAMENTS	NUMBER OF CASES IN WHICH MUSCLE FIBRILLA WERE OBSERVED	NUMBER OF CASES IN WHICH MUSCLE TWITCHING WAS SEEN	NUMBER OF CASES IN WHICH CILIARY MOVEMENT WAS SEEN.
Medullary cord ¹	90	19	71	35	.49	4	12	2
Branchial ectoderm	15	4	11	6	.55	.	..	7
Abdominal ectoderm	18	5	13	5
Axial mesoderm alone	30	8	22	1 ³	..	3-5 ⁴	..	.
Notochord	3	0	3
Endoderm (yolk)	2	0	2
Total	158 ²	36	122	42 ³	.	7-9	12	14

¹ In many of these cases no attempt was made to exclude all of the axial mesoderm. This accounts for the presence of muscle fibers in some.

² The excess of this number over the total number of recorded experiments is due to the fact that in some preparations several kinds of tissue were included.

³ This isolated case is one of a series in which the attempt was made to separate the myotomes from the medullary cord along their natural boundary. This is very difficult to do with absolute accuracy and it is supposed that in this case some cells from the medullary cord were left attached to the mesodermic tissue. In subsequent experiments cutting in close proximity to the nervous system was avoided and only the lateral portion of the mesoderm was taken.

⁴ The small number of cases recorded as showing striations is due to the fact that the preparations were examined only in toto. Had sections been cut it is believed that the number of positive observations would have been considerably larger.

only in certain cases that the oil immersion could be used, and then it was found to have no great advantage over the water immersion. A large number of sketches were made, nearly all with the camera lucida. In making these especial care was used to show the length of the fibers, and the form of the end organ correctly. Owing to the extreme fineness of the terminal filaments and the constant changes which they undergo, it is not, however, possible always to draw them with absolute accuracy in every detail. Still

is believed that any deviations which may have crept in have not misrepresented the essential character of the structures. The original sketches were made only in outline. The finished drawings, which are reproduced in the plates, were traced from these, details of texture being filled in in accordance with studies made for the purpose. Individual cells, when appearing by themselves, have been in most cases drawn in with the camera, but in indicating the larger masses of cells nothing more has been attempted than to give their general character. For instance, the exact arrangement of yolk and pigment granules was not copied because it was felt that this was not essential, and it would have required much time to the exclusion of the study of essential features.

Study of the material has been confined almost entirely to the fresh preparations. In fact it must be admitted that one serious defect in the work has been the impossibility of obtaining satisfactory preserved specimens. The ideal procedure would be first to study the growth of a particular fiber, recording the events by frequent sketches, and then to preserve that same specimen, demonstrating by suitable histological methods the structural identity between the fibers studied and the nerves found within the embryo.¹⁰ Owing to the extreme delicacy of the structures and to the almost fluid consistency of the lymph drops, it has, however, been impossible to do this, since the mere immersion of the preparation in any fluid brings about a disarrangement of the tissue, and in many cases the clot with the implanted tissue becomes loosened from the cover, or the tissue falls out of the clot. The method which has given the greatest promise is fixation in osmic acid vapor with subsequent hardening in Tellyesniczky's bichromate acetic mixture, and staining in alcoholic haematoxylin by the method of Oskar Schultze '04. In some of these preparations

¹⁰ Since this was written Dr. M. T. Burrows of the Rockefeller Institute, while working with me has devised a satisfactory method for obtaining permanent preparations. He has shown that embryonic nervous tissue of the chick, when isolated in the proper medium, gives rise to the same long filamentous processes as does that of the frog; and further, that by staining the preparations in Held's molybdenum haematoxylin the neurofibrillae in these filaments are brought out very clearly. An account of this work will be published at an early date.

isolated cells of various kinds have been well preserved (fig. 12) but satisfactory preparations of the nerve fibers have not been obtained. Some of the preparations have been cut into serial sections. Nerve fibers were found within them, but in all cases they were broken off at the surface of the tissue.

This defect in method has in a measure been offset by the experiments described elsewhere ('10) in which the nerve fibers from the medulla oblongata were shown to have grown into a blood clot implanted in their path.

General description of material

The developmental processes which have been observed in specimens prepared as described in the last section involve only the histological differentiation of the tissues. The gross morphological changes have no resemblance to those which take place within the embryonic body. This is as might be expected even on purely mechanical grounds, for the stresses and strains which are brought to bear upon the developing organs when enveloped in the fibrin must be entirely different from those within the intact embryo.

From the time when the tissue is implanted in the lymph it shows a tendency to spread out (fig. 16), and often broad laminae made up of a single layer of cells (*l*) are found at the periphery of the mass, while individual cells may move off entirely by themselves. This is the case with both nervous and axial mesodermic tissue, as well as with pieces of ectoderm, though the latter more often roll themselves into complete spheres. One notable peculiarity that has frequently been observed is the formation of large round or oval openings in the flattened tissue (*fen*), which may be surrounded by very narrow bands or rings of tissue with cells sometimes in single file (*cd*). This phenomenon may possibly be due to the mechanical action of the fibrin upon the implanted tissue, but the spreading out of the cells into thin sheets seems to result largely from the activities of the cells themselves. These activities, which are common to several tissues, in fact to all except the very inert yolk-laden endoderm and, perhaps, the notochord, may be referred to a form of protoplasmic movement having its

seat in the hyaline ectoplasm found at the angles and sometimes at the borders of the cells. The movement cannot be observed clearly in the larger masses of cells on account of their opacity, but it may be seen very clearly in those cells which leave the main masses and wander off by themselves. These cells are irregular in shape, varying from unipolar to multipolar form and having a varying amount of ectoplasm at their angles (figs. 23 and 27). The movement is amoeboid in character and results either in a change in shape of the cells or in their movement as a whole (text fig. 2). Such cells are found usually in greatest numbers in preparations of the medullary cord, and it is here that they are most active, though cells from the mesoderm are often quite similar. However, it is only the protoplasm of cells from the medullary cord and from the cranial ganglia (branchial ectoderm), that gives rise by its movement to long fibers. Cells of the epidermis show their power of movement in somewhat different form. As has frequently been observed, the general tendency of isolated bits of epidermis is to round off into small vesicles, which, when left in water, may move about for days by means of their cilia. Within the lymph the same thing frequently takes place, although there is apparently greater resistance to the process of rolling up, and the cells may often remain together in the form of extensive sheets. Along the free border of these sheets of cells there often appears a fringe of hyaline protoplasm, which undergoes continuous amoeboid changes (figs. 13 and 14 *pl.fr.*) In one case of this kind it was observed that the sheet of cells gradually spread out toward the side on which this fringe was placed. Since the work of Peters ('85-'89) it has been generally admitted that wound healing in the epidermis is primarily due to the movement, in part amoeboid, of the epithelial cells, so that it seems quite possible that in this fringe of hyaline protoplasm above described, we have one part of the mechanism by which the movement of cells in wound healing is brought about. The most inert of all the tissues is the endoderm, which will remain for days in the lymph, practically unchanged, gorged with yolk and devoid of hyaline ectoplasm. The notochord is also very inactive, although large pieces of this structure may show after a time the early stages of normal differ-

entiation, unaccompanied, however, by growth, *i.e.*, increase in length.

The changes which take place through the protoplasmic activity of the embryonic cells can usually be distinguished from those which are due to the action of the clot or the sudden spreading out of the drop of plasma. Likewise the fibrin can readily be distinguished from the hyaline protoplasm of the cells, although even in the fresh specimen it varies considerably in appearance. Sometimes the fibrin filaments, in spite of their extreme fineness, are plainly visible, and in other cases there are comparatively few to be seen. They may be found singly or in bundles, and often run for a long distance in a straight line, or sweep around in circles, the individual filaments running from one strand to another. The threads are seen to radiate from the transplanted tissue, and often they may be traced from the hyaline ectoplasm of the embryonic cells, upon which they apparently exert considerable tension. This may result in drawing out the ectoplasm to a narrow fringe (figs. 9, 10, and 28), which differs, however, from the fringe of active protoplasm described above, in that it does not continually undergo changes in form. Evidence of still greater tension is found in cells which are drawn out into spindle shape, and which often seem to be pulled along bodily, as may be seen in figs. 9, 10 and 11 which show three successive views of the same cell (*ct₂*.) Sometimes long chains of cells in single file or slightly overlapping one another may be formed. Direct evidence of mechanical tension may be had in observations like the following: A long thin fiber-like structure was observed in a preparation containing branchial ectoderm extending, tightly stretched, from a pear-shaped cell to a mass of cells some distance away, when suddenly this strand of protoplasm broke, contracting into a short thick process which remained attached to the cell. Again very fine protoplasmic threads are frequently found spanning the round openings in the masses of tissues, which have been described above (fig. 16 *fil.*). These threads are always taut and are apparently due to the stretching of originally shorter protoplasmic connections between the cells, as the holes in the tissue enlarge.

Similar filaments are often found extending from one cell to another (figs. 17 and 27).

The histological differentiation of the tissue in successful preparations is specific and normal, although it does not proceed so rapidly nor become so complete as when the tissues are left in their normal environment. No doubt one factor which contributes to this retardation of development is the insufficient supply of oxygen within the moist chamber. This is indicated by the slow rate of absorption of the yolk, as compared with its rate of absorption in the embryo. It is only in those specimens that have been kept alive for a week or longer that there is any great diminution of the yolk contained in the cells. The most noticeable histological differentiations that have been observed in the various isolated tissues are the following: the formation of typically striated fibrillar substance in cells taken from the axial mesoderm; the development of the cuticular border in ectoderm cells and the growth of cilia which may continue in action for days; the formation of typical chromatophores, most probably from cells derived from the medullary cord; and the formation from the central nervous system and from the cranial ganglia, of the long protoplasmic filaments, which are identical with the nerve fibers of the embryo and which are the especial subject of the present investigation.

Muscle fibers have been found not only in cases where a portion of the axial mesoderm was left in contact with the medullary cord, but also where it was isolated entirely from all other tissue, showing that the cells of the muscle plates at this stage, *i. e.*, before visible differentiation has begun, have the power of self-differentiation in the highest possible degree. This is in conformity with the results obtained from the study of muscle tissues in embryos deprived of the central nervous system. It is of course only in certain favorable cases that the presence of muscles fibrillae can be observed in the fresh specimen, since the tissue, unless it spreads out, is too opaque to permit of satisfactory observation *in toto*. While it is only the first stages in differentiation that take place, the yolk never being completely absorbed, the most

characteristic structure of the muscle fiber, the striated fibril, is formed with the alternating dark and light bands as well as Krause's membrane plainly visible. In many specimens in which the myotomes have been taken out with the medullary cord, muscle twitchings have been observed, beginning to occur the next day after isolation, and continuing sometimes up to the sixth day. No contractions have ever been witnessed in muscle completely isolated from nervous tissue.

Characteristic pigment cells have been observed a considerable number of times to arise apparently from pieces of the medullary tube from which large numbers of single cells had separated. But only fragmentary information is at present available regarding the development of these cells. In one case, which was under observation for several days, it was found that the pigment first arose as a round mass of granules lying just to one side of the nucleus. This gradually increased in size and then the pigment granules became scattered through the cytoplasm. In the meantime the yolk was almost entirely absorbed. After the cells are fully differentiated, observations from day to day showed that the individual cells changed slightly in form (figs. 24-26, *a*). While the evidence is by no means conclusive, especially since no great care was taken to exclude the presence of mesoderm cells, the fact that pigment cells were frequently formed from pieces of medullary cord, suggests the possibility that these cells may normally take origin in part from this source, most likely from the ganglion crest. This suggestion is borne out by the fact that pieces of medullary cord or cranial ganglia when transplanted to various regions of the embryonic body often break down and give rise to large numbers of pigment cells.

The above observations are recorded here primarily for the purpose of showing that the mode of procedure employed in the experiments permits the characteristic differentiation of various tissues to take place. It is of importance to establish this fact in order that the interpretation which has been given the observations upon the behavior of nervous tissue under these conditions may not be called into question as being based upon something entirely abnormal. It must of course be admitted that some of

the phenomena which have been observed may not have their counterpart in the embryonic body, but fortunately the careful comparison with what takes place in the latter enables us to discriminate with a fair degree of accuracy between the abnormal and the normal.

Description of the behavior of nervous tissues

The early changes which isolated pieces of medullary cord undergo are not very different from those seen, for instance, in pieces of mesoderm. There is merely greater protoplasmic activity. This frequently results in the separation of numerous cells which may move off individually from the main mass of tissue (figs. 10, 11, 16 and 27), or it may result in the formation of sheets of cells, one layer thick, which form a more or less complete fringe around the main mass (fig. 16). The formation of rings, as described above, is also frequent. These changes begin on the day on which the tissue is implanted, and may continue for several weeks, although the first week—usually the first four days—witnesses practically all the essential changes that take place. Observed from time to time, these cells may be seen to change their shape and their position relative to one another. Frequently they show anastomoses, though in a great many cases apparent continuity is often found on continued observation to be merely due to very close contact which may later be relinquished.

The striking peculiarities of this tissue have never been observed earlier than on the next day after isolating. Then it is that the long filaments, identical in form with the nerve fiber of the normal embryo, begin to appear. Two and three days after implantation they show their greatest activity. After the fifth day they are usually no longer to be found.

It will be well to begin with a description of one of the most striking typical cases and to consider the more aberrant forms afterward. In the case chosen for this purpose (experiment 137) the tissue was isolated from an embryo of *R. palustris* and the lymph was taken from an adult *R. pipiens*. The day after the preparation was made, there appeared on one side of the main

mass of tissue a long stout process of hyaline protoplasm, which, when first observed, was about 90μ in length, and extended out from a tapering cell (fig. 7). Examined an hour and a half later, this process was found to be only a little (about 17μ) longer, but the form of the end had changed considerably (fig. 8). It could also be seen that there were two separate fibers instead of a single one, one partly overlapping the other. Eight hours and a half later the specimen was again examined and found to have undergone remarkable changes (fig. 9). No less than four fibers could then be distinguished diverging from one another in their direction of growth, and each with its characteristic branched end (*npl*) continually undergoing change in form. The longest fiber (*nf₁*) was about 220μ in length. At this time the preparation closely resembled the condition described above in the normal embryo (figs. 3 and 4). Another interesting and important feature shown by this preparation was the action of the fibrin (*thr*) upon certain of the cells (*ct₁*), and the independence of the protoplasmic filaments from the fibrin threads. Twelve hours later, on the following morning, the change noticed was again very striking (fig. 10). Two of the fibers (*nf₂* and *nf₃*) were branched and all had lengthened materially, the longest being about 480μ in length. The ends of the fibers continued to show the same activity as before. Throughout their entire length the fibers consist of hyaline protoplasm, with no yolk nor pigment granules whatever. Slight varicosities are present in places, and often the fibers show a faint fibrillation and sometimes are slightly mottled. The thickness of the fibers, $2-3\mu$, in this case is rather unusual. Changes in the cells are also important. The number of loose cells has increased, and they move along slowly from place to place, while changing their shape. Their movement is, however, entirely independent of the fibers. The tension of the fibrin filaments upon some of the cells is clearly shown. The cell (*ct₁*) noticed previously is very much drawn out as compared with its condition the day before. Eleven hours later still further elongation and branching of the nerve fibers is to be seen (fig. 11), the longest now being about 600μ . The loose cells are more numerous and some of the fibers are partly obscured by them. The exact

extent and manner of ending was therefore not observed in all fibers—*nf*₁, and *nf*₂, for instance, being only incompletely recorded. The cell to which the fibrin filaments were attached is now drawn completely out of the main cell mass. Twelve hours later the longest fiber is found to extend 557 μ beyond the point of ending the evening before, having thus grown at the rate of 46 μ an hour, the total length now being about 1.15 mm. Many branches are present and many new fibers are visible in the same region, but the proximal part of those described has become covered over by the loose cells which have wandered out from the main mass. These circumstances rendered it impossible to make further accurate observations of the changes which took place, and the specimen was therefore preserved. Unfortunately, the region which had been observed most carefully was completely disarranged in the course of fixation and the preparation in permanent form was almost useless for further purposes.

This same preparation showed a number of other fibers of interest. Among these was one which arose from a single isolated cell, and which was visible throughout its entire length (fig. 21). When first observed this fiber had a total length of 453 μ . At a distance of 303 μ from the cell it bifurcated, the longer branch being 150 μ , and the shorter, which afterward grew to be the longer, 107 μ . At this time, the ends were not very active and that of each branch was almost globular, with but one blunt pseudopodium. The cell itself was unipolar. Examined at the expiration of four hours and three quarters (fig. 22), the change in the fiber was found to be very great. The cell itself was unchanged but the fiber then had a total length of 631 μ , and one of the original branches had again bifurcated. All three of the terminal enlargements were exceedingly active, and all were provided with a number of fine filaments. The increase in length from the cell to the tip of the longest branch was 221 μ , which is at the rate of $.77 + \mu$ per minute, or 46.5 μ , per hour. Comparison of the two stages shows that the greater part of this was due to terminal growth, but the distance between the cell body and the first bifurcation increased 21 μ , and the curvature of this part of the fiber was partially straightened out.

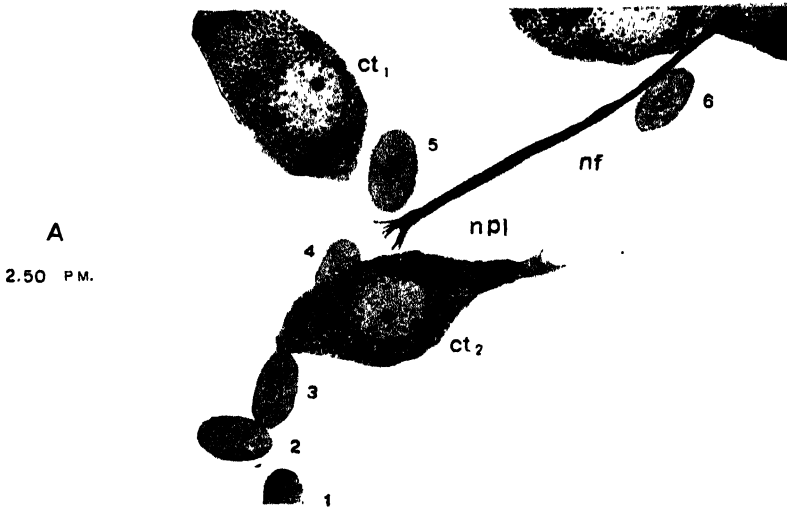


Fig. 2 Three views of a growing nerve fiber, observed alive in a clotted lymph preparation. 1, 2, 3, 4, 5, red blood corpuscles in fixed position; *ct₁*, and *ct₂*, single cells which were seen to wander across the field; *nf*, nerve fiber; *npl*, growing end of motile protoplasm. $\times 420$. A, As seen at 2.50 p.m., two days after isolation of the embryonic tissue. B, As seen at 4.40 p.m., the same day. Note change in form and position of the loose cells. C, As seen at 9.15 p.m., the same day. Movement of cells has covered over the proximal part of the fiber.

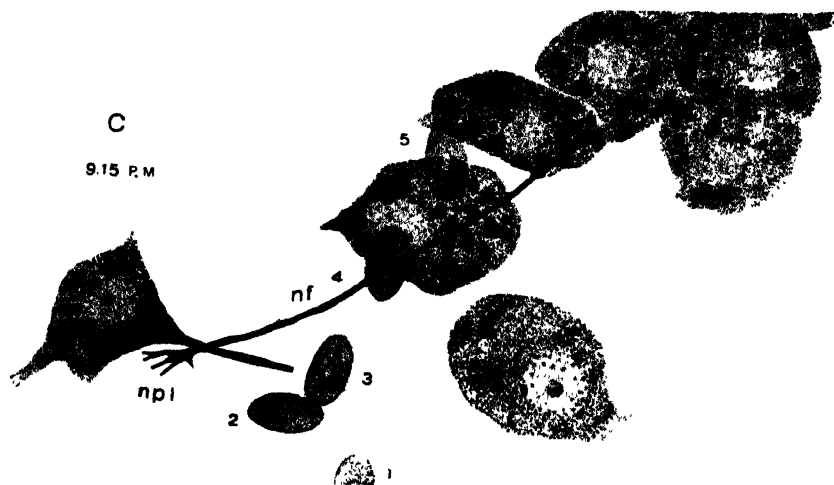
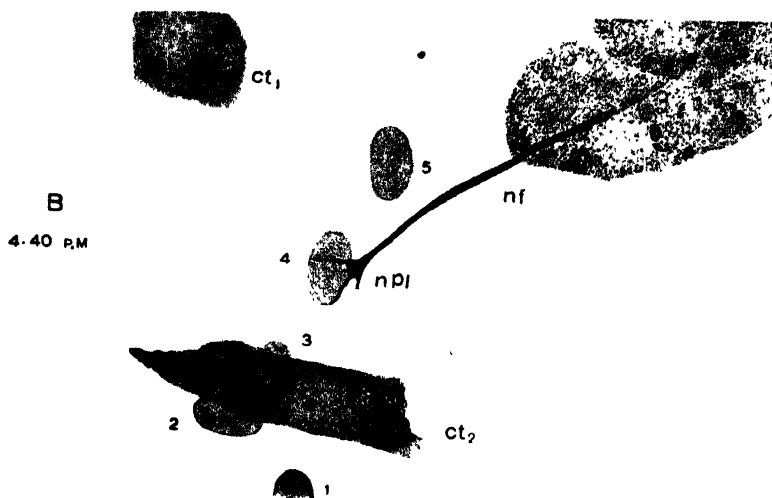


Fig. 2 (Continued)

Still another feature of importance was shown in the preparation under consideration. In the previous observations upon the growing filaments, it had been impossible to find absolutely fixed points from which to measure their increase in length, so that there remained the possibility that the extension of the fibers was not due to the active movement of the enlarged end but rather to the passive shifting of the cells from which the fibers were seen to arise. While such an objection would not hold in cases like the one just described, where the fiber is visible throughout its entire length from the cell of origin to the amoeboid end, such instances are not very common, and it is desirable to have other means of determining which structure is moving. The specimen under consideration has demonstrated that in good preparations where the clot is firm, the red blood corpuscles, some of which are nearly always to be found mixed in the lymph, can be used for this purpose. One particular case (text fig. 2) will serve to illustrate the point clearly. A short stout fiber (*nf*) was observed emerging from a mass of cells, its cell of origin not being visible. Near the end of the fiber (*npl*) was a group of five red blood corpuscles (1-5) arranged characteristically, and at the point of emergence of the fiber was another single corpuscle (6). In the vicinity of the end of the fiber were two large separate cells (*ct*₁, and *ct*₂) derived from the implanted tissue. The relative position of the structures just mentioned is shown in fig. A. The end of the fiber was then only fairly active. An hour and fifty minutes later (text fig. 2 B) it had progressed only 25 μ , as measured by its relation to corpuscles 4 and 5. The loose cells (*ct*₁ and *ct*₂), however, had moved considerably, but in a direction approximately at right angles to the direction taken by the fiber, showing that they could not have been concerned in the movement of the latter. At the same time these cells had changed their shape materially, as is shown especially in cell *ct*₂, this change in shape being undoubtedly due to the activity of the hyaline ectoplasm. Six hours and twenty-five minutes after the first observation was made (text fig. 2 C) the distance through which the end had moved was 100 μ , i.e., at the rate of 15.6 μ per hour. This rate is slow as compared with that observed in other cases, though it is considerably faster

in the first interval than in the second. Considerable numbers of loose cells had by this time moved into the field, so that the proximal part of the fiber, which was visible earlier, was then covered up, but the five corpuscles were still plainly in view and their relative position remained still unaltered. During all this time the end of the fiber had been changing its form continually. It is inconceivable that the fiber could have been pushed past the corpuscles by any force acting from behind. Again, it is impossible that the five corpuscles could have shifted materially and at the same time have retained their same relative position. Nor can we account for the movement by tension upon the fiber from beyond, for such tension would act also upon the corpuscles and upon the cells in like manner; and the continual change in the form of the end and the lack of any appearance of tension likewise speak against this mode of accounting for the movement. We therefore cannot escape the conclusion that the extension of the fiber is due to the activity of the enlargement at its end.

The character of the movement that takes place at the end of the fiber is difficult to describe. The filaments in which the fiber ends are extremely minute and colorless, showing against their colorless surroundings only by difference in refraction. The eye perceives, therefore, only with difficulty an actual movement, though when an active end is observed for five minutes it will be seen to have changed very markedly, so that in making drawings one encounters the difficulty of having the object change before the outline can be traced. In order to show the change of form that does take place, a series of sketches are reproduced in text fig. 3, which show the same fiber at intervals of from five to nine minutes. The specimen was a portion of the ectoderm with the underlying cranial ganglia taken from the branchial region of a *sylvatica* embryo. When first seen the fiber was about 450μ long. The next day, April 6, 1909, four days after the preparation was made, the fiber had increased in length to 800μ when the first sketch was made at 10.50 a.m. The changes shown in the sketches took place between 10.50 and 11.37 a.m. The end of the fiber was just beyond a red corpuscle, the position of which was fixed. This is shown in outline in each figure. It will be

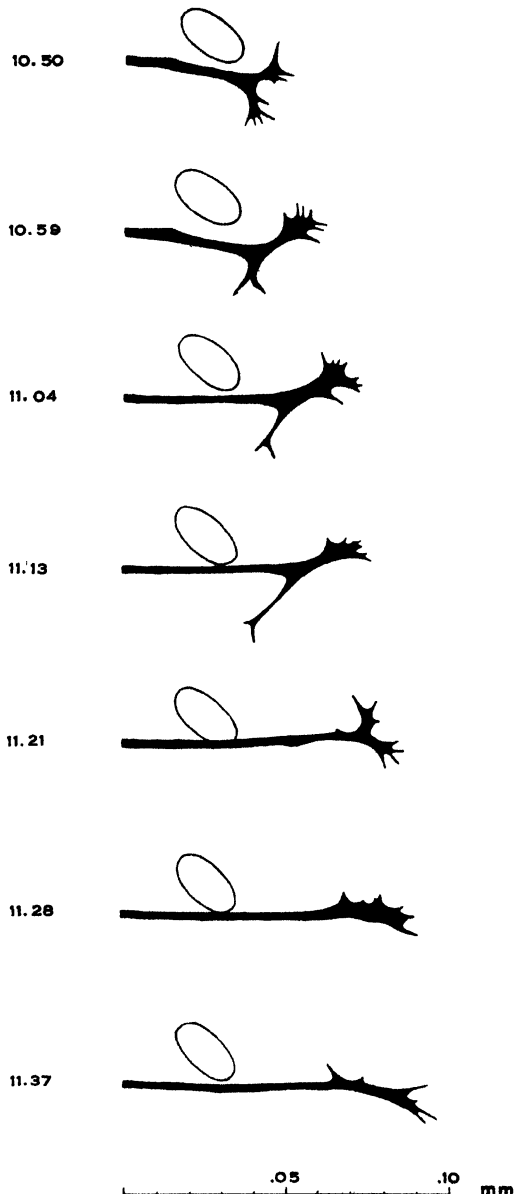


Fig. 3 Seven successive views of the end of a growing nerve fiber showing its change of shape and progression. The sketches were made with the aid of a camera lucida at the time indicated on the left. The red blood corpuscle, shown in outline, marks a fixed point. The observations were made upon a living preparation of ectoderm from the branchial region, isolated in lymph, four days after isolation. The total length of fiber at that time was 800μ .

seen that during the time in which it was under observation, new processes or pseudopodia were formed and some present at first were withdrawn. The movement of the end was 44μ during the 47 minutes in which it was observed. This is at the rate of $.94\mu$ per minute or 56μ per hour, which is the most rapid extension that I have ever observed. This is all the more remarkable because of the great length of the fiber, for one would naturally expect a gradual lessening of the activity as the limit of growth is approached. There can be no doubt that the red blood corpuscle was a stationary point. Its position with reference to five other corpuscles remained fixed throughout the period of observation, and even on setting the preparation on edge, *i.e.*, turning it 90° , no change in relative position was observed, although the plasma in the meshes of the clot flowed perceptibly. The direction of growth of the fiber served, however, to draw the fiber close to the corpuscle. One feature shown in the present case and observed also in a number of others, was the formation of processes of considerable thickness and length which were afterward withdrawn (text fig. 3), indicating that the movement of the end is not directed constantly toward a particular goal."¹¹

As already mentioned, the fibers that are found in preparations of the nervous system usually proceed from masses of cells so opaque that their exact mode of origin cannot be determined. Fibers whose origin could be seen in single cells have been observed a number of times and several are shown in figs. 18 and 20. Fig. 18 differs from the others in so far as the cell itself has wandered out from the mass, remaining connected with the latter by a long filament (*b*) about 300μ long. The hyaline process (*a*) at the distal end of the cell is relatively short.

This leads to the consideration of the formation of protoplasmic fibers by the drawing apart of cells. Fig. 19 shows a bipolar cell the processes of which were formed in this way. They are tightly stretched and at both ends terminate in masses of cells, so that it is not possible to make out their exact mode of termina-

¹¹ This is akin to what Held has termed "Prinzip der Auswahl" for which he has adduced evidence (*Op. cit.*, p. 270).

tion. The cell resembles strongly a spinal or cranial ganglion cell, and as a matter of fact this particular one was derived from a piece of branchial ectoderm, but the case is not specific, for similar cells have been found in other tissue, even in pieces of muscle plate, and drawn out fibers of this kind have been observed in large numbers of cases, though more frequently in nervous tissue than in any other. They demonstrate the great extensibility of the embryonic protoplasm and show how a nerve fiber may be passively drawn out to enormous length, as no doubt occurs in the embryonic body, for instance, in the case of the *r. lateralis vagi*.

Frequently large numbers of cells become loosened from the main mass and scatter themselves in the periphery. Such cells may remain separate or they may often be connected with one another by hyaline protoplasmic filaments (fig. 27). In many cases, however, the connections are more apparent than real, as cells that seem to have been joined will frequently glide apart and demonstrate the supposed continuity to be merely a close contact.

In a few cases the long nerve fibers have apparently formed distinct nets. One of the most remarkable observed is shown in fig. 29. There are a large number of free endings in the group, as well as many apparent anastomoses. This specimen was observed late one evening, and while it was under observation one of the connections (*x*) was actually resolved. On the following morning it was found that, with two possible exceptions, all of the anastomoses had been severed, each fiber being independent of the others. Many of these apparent protoplasmic fusions were obviously to be accounted for by our optical limitations. The protoplasmic filaments are very delicate, colorless, and without visible limiting membrane. When two such structures touch one another, an appearance of fusion is readily given and one must be extremely cautious in interpreting observations. On the other hand, there are undoubted instances of strong adhesion between the cells by means of filaments, as shown by the tension upon them if the cells move apart, but it must be borne in mind that very slight differences in the physical properties of the protoplasm of which the cells are composed would suffice to permit

or prevent fusion between contiguous elements, and the same elements might therefore at one time be separate, and at another continuous.

In some cases large numbers of the protoplasmic fibers have been found matted together, in an inextricable tangle (fig. 17). These have undoubtedly been derived from groups of cells, and the specimen reminds one of the condition that is found after the medullary cord of the embryo is removed from the trunk region, when the fibers from the brain grow out in a large bundle and lose themselves ultimately in the mesenchyme.

DISCUSSION OF RESULTS

The significance of the experiments in the interpretation of normal development

In attempting to estimate the significance of the foregoing experiments as elucidating the processes of normal development, we are at once confronted with the question whether the conditions in the experiments are sufficiently like those in the embryonic body to warrant any comparison at all. This can be answered most satisfactorily by carefully comparing the activities of isolated tissues with the activities of the tissues in the normal embryo. Such an empirical determination must have more weight than any amount of *a priori* argumentation upon the subject. The phenomena which can be compared and interpreted most readily are those of movement and of tissue differentiation.

The movement of the embryonic cells in the lymph clot is very distinct, and is due beyond doubt to the activities of the hyaline ectoplasm (figs. 23 and 27), which is accumulated especially at the angles of the cells. It there forms extremely fine filamentous pseudopodia, through the activity of which the cells may change their shape or move from place to place. The exact character of the movement is not the same in all kinds of cells and it varies greatly in intensity. Axial mesoderm and medullary cord yield cells that frequently wander for considerable distances by themselves; epidermis, when it does not roll up into bands or spheres,

may form a hyaline fringe (figs. 13 and 14), and spread out considerably; pieces of the central nervous system and the primordia of the cranial ganglia give rise to the fiber-like structures described in the last section; the endoderm and notochord remain almost inert.

It is, of course, needless to point out here the wide occurrence of protoplasmic movement in the normal development of organisms, and it will suffice to mention a few special cases which bear more directly upon the present problem. Within the body of the vertebrate embryo at the stage of development under consideration there is ample evidence that this kind of movement takes place. It is then that the mesenchyme is beginning to form by the breaking down of the epithelial mesoderm and the shifting of its cells to regions far removed from their source; and similarly the cells of the ganglion crest leave their place of origin and wander for a considerable distance before grouping themselves together as the spinal ganglia. In these cases we are dealing largely with the movement of single cells. A notable example of the active movement of masses of cells is afforded by the lateral line rudiment, which, in the course of several days, extends all the way from the head to the tip of the tail, as experiments show, by its own motile force (Harrison '03). At a later period of development after the first nerve trunks are laid down, there is an actual movement of Schwann cells along the nerve fibers, as I have been able to observe in the tail fin of the living tadpole ('04). In the same object Clark ('09) has watched the growth of lymphatics by sprouting at their ends. This observer has not only seen the actual amoeboid movement of the endothelial cells, but has also been able to show that the movement may be stimulated and directed by definite bodies, such as, extravasated red blood corpuscles. Within the central nervous system there is undoubted shifting of groups of ganglion cells, such movement having been taken into account by Cajal ('92, '99) in his original hypothesis of chemotaxis, and more recently by Kappers ('08) in his papers on neurobiotaxis, though it must be admitted that the evidence for active movement in the two last cases is merely inferential. In the closure of wounds we have another example

of the amoeboid activity of cells, as was first pointed out by Peters ('85, '89) in the cornea of the frog, and the observations of Barfurth ('91) and Born ('96-'97) on the epidermis of amphibian larvae and embryos confirm this view. More recently Eycleshymer ('07) has observed directly the movement of epidermal cells over a denuded wound surface in *Necturus* embryos.

The phenomena of movement, which may be observed in the embryonic cells isolated in lymph, must, in view of the above considerations, be considered as manifestations of activity similar in kind to those shown by cells within the normal embryo. The differences which may exist are unimportant for our present purpose. For instance, the peculiarities of form (fig. 6) assumed by the larger masses of cells when transplanted, are not to be taken as an index of a marked abnormality of conditions which might introduce entirely new features into the movements of individual cells, for these strange formations may be accounted for by the peculiar mechanical conditions obtaining in the clot. Analogous deviations from the normal in the gross form of parts are found, accompanied, however, by normal differentiation of tissues, when pieces of the medullary cord are transplanted to strange regions within the body of the embryo.

The phenomena of differentiation permit the drawing of a much closer parallel between the behavior of the tissues in their normal environment and when isolated in lymph than do the motor activities. Each type of cell follows the same course of differentiation which it would have taken had it not been removed from the embryo, as is seen, for instance, in the formation of striated fibrillae in cells from the axial mesoderm, a cuticular border in cells from the epidermis, and typical chromatophores from the walls of the medullary tube. Ciliary activity, which may persist for days in the case of tissue from the medullary cord or the ectoderm, and muscle contractions, which occur in the muscle plates when transplanted along with parts of the central nervous system, bear witness to the possibility of normal functioning under the conditions of the experiments.

It is seen from the above that the behavior of embryonic cells when transplanted to lymph is specific as regards the character

and degree of their motility, the quality of their differentiation, and their mode of physiological activity when differentiated. Even had we but scant knowledge of the normal development of nerve fibers, we should therefore be justified in concluding that the phenomena witnessed in preparations from the central nervous system are a representation of what occurs when the nerve fibers develop in the body of the embryo. The study of normal development strengthens this conclusion immeasurably by revealing, in the young stages of peripheral nerves, structures which are strikingly similar to the protoplasmic fibers found in the lymph. (Cf. figs 4-6 with figs. 8, 9 and 21). Such fibers with their active amoeboid ends are formed by cells taken both from the walls of the medullary tube and from the rudiments of the cranial ganglia, and must be regarded as specifically nervous. It is thus scarcely conceivable that the identity between the fibers found in the lymph and true embryonic nerves can be questioned, for that conclusion, as just pointed out, is based not only upon the general fact that the differentiations taking place in the isolated tissues are normal and specific, as far as they go, but also upon the particular morphological resemblance between the structures in question and undoubted nerves. It must be admitted that the case might be made even stronger were it possible to preserve the isolated nerves satisfactorily and stain them by the specific stains for neurofibrillae, though this is in no sense essential to the argument here advanced (see footnote, p. 807).

The bearing of the experiments upon the theories of nerve development

As the experiments clearly show, one of the fundamental characteristics of the neuroblastic protoplasm is its high degree of motility, which, being manifested by only a limited portion of the cell, results in the drawing out of the protoplasm into a long filament representing the axone of a nerve fiber. The extreme tip of the fiber, which is the *cône d'accroissement* of Ramon y Cajal, remains remarkably mobile, while the body of the fiber evidently soon acquires a firmer consistency and considerable tensile strength.

The latter is probably the result of neurofibrillation, since, as the work of Held and of Ramon y Cajal shows, the neurofibrillae extend almost to the tip of the fiber even in very young nerves.

The movement of the neuroblastic protoplasm, which is brought about not by passive extension but by its own activity, will take place in a medium foreign to the embryonic body, and there can here be no possibility either of accretion by transformation of living protoplasm already *in situ*, or of outgrowth of fibrillar substance within such protoplasmic connections, since there is nothing of the kind present, the solid parts of the culture medium being nothing but fibrin. Quite aside from this consideration, the character of the movement, as observed directly, precludes all possibility of extension according to the conception of either Hensen or Held.

The criticisms of this conclusion that have appeared up to the present time, have been directed against my preliminary notices, in which the data were very briefly recorded and illustrations were few. Those who have expressed themselves adversely to the claim of conclusiveness are Hensen ('08), Schaeppi ('09), Kerr ('10) and Held ('09). Hensen, however, raises no specific objection and Schaeppi¹² is merely not convinced that the actual growing end of the nerve fibers was observed. The criticism of Kerr ('10) is more specific, though of the same kind as that of

¹² Schaeppi's criticism is directed mainly against my earlier experiments (Harrison '06), which had not then been published in full. I regret that through inadvertence no notice was taken of Schaeppi's appreciative though adverse critique in my full paper (Harrison '10), in which the logical bearing of the various experiments is set forth at some length. No claim of absolute rigorousness of proof for the non-participation of protoplasmic bridges in the formation of nerves, was there made for any particular experiment, except in the case of the one in which the nerves grew within the implanted blood clot; and even in this case it was admitted as a remote possibility that the embryonic cells which rapidly organize the clot might form protoplasmic bridges. Taken together, however, these experiments afford a mass of evidence against the protoplasmic bridge theory, which to my mind far outweighs that which has been brought forward in its favor. The experiments certainly rob the theory of any claim upon functional activity as a factor in the early development of nerves, even though the experiments with acetone-chloroform are not admitted as evidence. Turning now to the criticism of the present experiments, I feel confident that if Schaeppi had had before him the figures which I am able to present here, he would hardly have asked: "Wer in aller

Schaeppi. He has urged that in the lymph experiments the excised fragments may have included protoplasmic bridges such as he has figured from *Lepidosiren* embryos, and that these might differentiate later into nerve fibers. In reply to this it may be pointed out that the embryos used in the experiments were of a relatively younger stage than those Kerr has in mind and contained no protoplasmic connections of the kind figured, as serial sections readily show.¹³ Furthermore the pieces of the tissue were cut out with sharp scissors, sometimes by a single clean cut between the neural tube and the notochord, or in other cases, after the neural tube had been separated by the scissors from the muscle plates. All protoplasmic filaments must have been severed by this mode of cutting, and while, of course, short ends may possibly have been left attached to the cells, and have remained invisible in the preparations, they would by no means be able to account for the great length—over a millimeter in some cases—attained by the fibers. Besides, what is much more conclusive, *the end of the fiber is an actively motile mass of protoplasm.*

Held's ('09) criticism takes a different turn. He admits¹⁴ that the fibers seen in the lymph are really the beginnings of nerves (*Ansätze einer Nervenbildung*), though on the next page he maintains—on what grounds it is not clear—that if the nerves of an embryo did develop exclusively in the manner described by me,

Welt will mich denn davon überzeugen, dass das, was unter dem Mikroskope als das Ende einer Faser erscheint, nun wirklich in Tat und Wahrheit das Ende ist?"

In doubting that such ends, in which motion and extension can be observed with absolute certainty, are actual ends, it seems to me that the supporters of the protoplasmic bridge theory are pushing skepticism about one thing beyond the utmost limit, and at the same time are placing an equally unbounded faith in the invisible. One cannot but think of the epithet "*noli me tangere*" by which Hensen designated the outgrowth theory, and wonder if it might not be applied with much greater appropriateness to the plasmodesm hypothesis. Could the botanist, if pressed for an absolutely rigorous proof that the roots of a plant grow out from the radicle and are not preformed in the soil, give an answer based on evidence of any different kind from that given here for the outgrowth theory of nerves?

¹³ To my mind the protoplasmic bridges which Kerr figures are simply the processes that have grown out from the cells in the ventral part of the cord.

¹⁴ *Op. cit.*, p. 260.

they would after a time degenerate as do those which develop sporadically in the ventricular fluid. It is quite true that the nerves which grow out into the lymph-clot do ultimately shrink and disintegrate, but it is purely gratuitous to assert that this is *in consequence* of their original mode of growth, when it is in all likelihood due to the effect of continued unfavorable surroundings upon their subsequent development. Held also intimates that the nerves grown in lymph are incapable of functioning, though no sufficient ground for this statement is offered. But when he says "Die Beobachtungen Harrisons zeigen . . . mit welcher *Energie die neurofibrilläre Zellsubstanz aus der fibrillogenen Zone des Hisschen Neuroblasten hervorwächst,*" and when he says further on "Dass die fraglichen Experimente . . . die *elementare Bedeutung der Hisschen Neuroblasten* für die von ihnen herausgehende und vorschreitende Bildung der spezifischen Substanz des Nervengewebes illustrieren," then I can only express my cordial agreement, since in these sentences Held practically admits all that I have ever claimed for the experiments, viz: that they show the nerve fibers to be the product of the neuroblasts, and to be capable of being formed without the aid of protoplasmic bridges.

It would seem from the above that Held and myself were in pretty fair agreement regarding the question at issue, and I shall endeavor to show below just how our views are related, but it will be necessary first to consider the important difference that appears in the next following paragraph of Held's work, in which he maintains that the histogenetic study of the embryo shows more than the experiments, since it reveals the presence of a connective substance between the individual cells and organs of the body, which is used in the formation of the definitive nerve paths.

Held's sections are of exquisite beauty and show beyond doubt the structures he has described, but they fail on the other hand to prove that the same have any *essential* connection with the formation of the nerve paths. While it is true that the developing nerves seem to be very intimately joined with the protoplasmic bridges which Held describes, the very ubiquity of the latter in the embryonic body precludes the possibility of proving, by the

study of normal material, however clearly stained, that they are essential to nerve building. So long as we keep an animal in pure air without ever varying its surrounding medium, we have no means of knowing whether the nitrogen constituent is essential to its life or not. It is only by eliminating or at least by varying this part of medium that it can be shown not to be necessary. In the embryonic body, according to the descriptions of Held, no nerve can grow along a normal path without coming into intimate contact with the protoplasmic bridges or the protoplasm of the cells within the central nervous system. Until these are eliminated or modified, therefore, we can have no knowledge whether they are essential to the growth of the nerves or not. This is the crux of the whole question and it is this that the present experiments have settled, adversely to the view taken by Held, by substituting for the supposedly essential protoplasmic bridges, unorganized fibrin threads, which afford merely mechanical support to the growing nerves. In view of this I find it altogether impossible to accept Held's conception as correct, though just to what extent the protoplasmic net work which he describes may influence mechanically the growing fibers remains problematical, there being no ground for denying it a place as a subsidiary factor along with the other structures of the embryonic body. Notwithstanding this difference of opinion I think that it will become clear from the following, that, in the main, the relation between Held's work and my own is not antagonistic but complementary.

The elementary factors of nerve development

When we consider the elementary phenomena of nerve development in the light of the present experiments and of recent histogenetic studies, the first thing that stands out is that two separate processes are involved: the one is the protoplasmic movement, which results in the drawing out of a part of the neuroblast into a thread of protoplasm, the primitive nerve fiber; the other is the differentiation of this protoplasm by the formation within it of neurofibrillar substance. These may be referred to as the motor and the differentiation phenomena respectively. The existence of the former has either not been recognized or has been openly

denied by the adherents of Hensen's theory. Early protoplasmic connections have been observed by them, and by assuming these to be primary, as Kerr has done in the case of the motor roots of *Lepidosiren*, or at least by not realizing that connections of that kind are established by the outflowing of the protoplasm of the neuroblasts, they have been advanced as evidence in support of the protoplasmic bridge theory. Held and Paton, in their recent work, have emphasized the differentiation phenomena, and have totally failed to recognize the importance of the protoplasmic movement which precedes. This is of course a natural consequence of relying entirely upon specific staining methods. Means of demonstrating neurofibrillae at the earliest possible moment were sought and found, and though undoubtedly one result has been a great advance in our knowledge of the processes of neurofibrillation, it is equally without doubt that another result has been the production of a one-sided view of the development of the nervous system. This is exemplified in the opening sentence (following the historical sketch) of Held's monograph where one reads¹⁵ "Bei der Frage nach der Entwicklung des Nervengewebes handelt es sich um den Nachweis der ersten histologischen Charakteristika des späterhin so eigentümlich ausgeprägten und im Tierkörper weit verbreiteten Nervengewebes" (meaning by this the neurofibrillar substance). Held, it is true, holds that the fibrillar substance is formed by the neuroblasts of His, and he describes the pushing out of the fibrillae from the center into the peripheral protoplasmic net work; he also enters upon a discussion of the influences which bring about the nervous connections found in the adult organism,¹⁶ so that there is a close

¹⁵ *Op. cit.*, p. 10.

¹⁶ Held recognizes a number of principles in the outgrowth of the fibrillar substance which are closely similar to those stated by His and followed here, as, for instance, "das Prinzip der Achsenstellung" and "das Prinzip der Wegstrecke" (*op. cit.*, p. 278); and in his reference to chemotaxis (p. 148), and to the "Prinzip der Auswahl" (p. 270), Held's views lean strongly toward those of Ramon y Cajal. It must be borne in mind, however, that in all these cases Held refers solely to the movement of the *fibrillar substance* within the preformed reticular protoplasm, instead of recognizing that it is a mass of undifferentiated neuroblastic protoplasm that moves, and that this afterwards forms the neurofibrillae within itself by differentiation.

analogy between his view and the one advocated here. It is, however, the peculiar relation of the outgrowing nerve substance to the intercellular net work to which Held attaches the most fundamental importance, and it is in this respect, as comparison of his work with that of Ramon y Cajal shows, that he goes further than the methods he has employed would, in my opinion, warrant. What Held's figures really show, objectively expressed, is that the neurofibrillae are laid down within protoplasm and that they are always connected with the neuroblast, extending further peripherally in later than in earlier stages of development. This is in no wise incompatible with the results of my experiments, for the only difference concerns the source of the protoplasm in which the fibrillae develop, Held holding that it is formed of cells scattered all through the embryonic body, while I maintain on the basis of the experiments, that it flows out from the central cells, and thereby establishes the paths in which the neurofibrillae are formed. But it is this laying down of the primary nerve paths by means of a form of protoplasmic movement, rather than the process of neurofibrillation, that constitutes the specifically intricate problem in the development of the nervous system. The differentiation phenomena are naturally of great interest too, but though chemically specific, they are essentially of the same class as the differentiation phenomena witnessed, for example, in muscle or in the connective tissue cell; and they are not in any way comparable, as regards complexity of special relations, to the phenomena of the establishment of the primitive nervous connections by the outflow of the neural protoplasm. In order to discover the factors which influence the formation of the nerve paths, we must, therefore, in the first instance take into consideration this property of protoplasmic movement. This is of the utmost importance, and any theory of nerve development which fails to do so is sure to be misleading.

Analysis of the factors which produce the specific arrangement of the nerve paths

In proceeding with the analysis of our problem, the first point to consider is the localization of the energy which produces the outgrowth. The experiments answer this clearly: the primary act of extension by which the protoplasm of the neuroblast is drawn out into a fiber, the primordium of the axone, is due to forces immanent in the neuroblast itself at the time when outgrowth begins and probably for a considerable time before. This conclusion is based upon the direct observation that the movement takes place in the protoplasm itself without the application of any external physical force, and it is corroborated by the fact that outgrowth occurs even when the normal surroundings are radically modified, as in the present experiments or as Lewis ('07) and myself ('06, '10) have previously shown. That the original direction taken by the outgrowing fiber is already determined for each cell before the outgrowth actually begins, so that when it does begin it is dependent upon forces acting from within, follows first from the fact that the nerve fibers within the embryo tend to grow out in a given direction even when quite different surroundings are substituted for the normal, and secondly from the fact that the nerve fibers which grow into the clotted lymph, are there surrounded on all sides by an isotropic medium, which cannot conceivably be held to produce movement in a definite direction.

The formation of the protoplasmic nerve tracts falls, according to the foregoing, within Roux's definition of self-differentiation, by which is meant, not that the process is entirely independent of external conditions, but simply, as Roux ('85) in first defining the concept pointed out, that the changes in the system, or at least the specific nature of the change, are determined by the energy of the system itself.¹⁷ In the particular case in question this means that within a medium compatible with the life and growth of the neuroblasts the formation of nerve fibers may take

¹⁷ *Op cit.*, p. 423. In the "Gesammelte Abhandlungen," p. 15.

place without the application of any further external force either as stimulus or as motive power.¹⁸

The experiments indicate that some solid support is one of the essential conditions of growth, the fibrin threads apparently affording this support in the experiments with clotted blood and lymph. At least it has not been possible to induce growth in purely fluid media, and I am therefore inclined to hold to the hypothesis stated in the beginning (see p. 800), that some form of stereotropism plays a rôle in the outgrowth of the fibers, as Loeb

¹⁸ Miss Shorey ('09) objects to calling this self-differentiation on the ground that the lymph used in the experiments contains the products of metabolism of various organs of the body, including organs such as muscles whose physiological activities are similar to those of the embryonic parts which the nerves in question would normally innervate; and these products of metabolism are the substances which, according to Miss Shorey, stimulate the growth of embryonic nerves. While it is of course true that lymph is a complex medium and may contain a variety of such products, it is nevertheless pretty hazardous, to say the least, to assume that they are the same in the lymph of an adult frog as in the interstices of a young embryo, and, in the absence of experimental evidence, it is entirely without foundation to assume that it is these particular products that stimulate the neuroblasts to grow out. In my opinion, the lymph is merely a medium in which these structures are capable of growing as a mouse grows in air or a fish in water. There is no specific relation between these media and the kind of organism that develops. Roux had such cases in mind in casting his definition of self-differentiation. Within the lymph the growing nerve fiber is bathed on all sides by the same medium and it is impossible therefore for the latter to exert any directive action. In interpreting her own striking and very important experiments Miss Shorey has ignored a number of facts, brought out by Lewis ('06 and '07) and myself (Harrison '06), which are opposed to her theory, as, for instance, the experiments in which part of the medullary tube was removed, with the result that the longitudinal fibers from the remaining part grew straight out into the mesenchyme. Since these fibers would normally have grown within the substance of the cord, and since, to carry out Miss Shorey's hypothesis to its logical conclusion, it must be supposed that that tissue emits some product of metabolism capable of stimulating the growth of the intrinsic nerves, we should consequently find in the experiments the self contradictory condition of nerve fibers growing directly away from that particular tissue, the remaining part of the neural tube, which alone should be stimulating growth toward it.

Miss Shorey's experiments show that the extirpation of peripheral material has a marked effect in partially inhibiting the development both of the nerves supplying it and of the central neuroblasts themselves. It is significant that in these experiments the effect of removal was found to be much more marked after a considerable time had elapsed, and also that in no case was the development of peripheral nerves and their neuroblasts entirely suppressed. This indicates that the

('98 and '07) has thought to be the case in the regenerative growth of epithelium. This, however, would not necessarily preclude the possibility of the bridging of very small spaces by the outgrowing fiber.¹⁹

There are cases which have been described where nerve fibers are found extending for considerable distances in the ventricular fluid, as for instance Reissner's fiber, and all those cases which Held refers to as developmental curiosities, but these do not contradict the view here advanced, for it is quite possible that the fibers originally grew along the surface of the lining of the neural tube. I am also of the opinion that the case described by me in which a nerve was found crossing the peritoneal cavity is not altogether against this view, because the attachment of the nerve was probably effected before the separation of the splanchnopleure and somatopleure took place.

Given a form of protoplasm with power to extend itself in a definite direction so as to form a fiber, the next step is to determine the influences which may modify the direction of its growth and produce the specific arrangement of nerve tracts found in the mature organism. His ('88) was the first to show that developing nerves in the normal embryo begin their growth in a straight line, and that this direction may afterward be modified by various agencies such as the shifting of parts, or by meeting obstacles in the path of growth. The normal amphibian embryo affords abundant confirmation of these observations which His made upon the human embryo. The peripheral fibers from the dorsal nerves of Rohon-Beard, for instance, run at first laterally in a straight line through the notches between the muscle plates; they soon reach the epidermis and are there deflected through an arc of nearly 90°, to a dorso-ventral direction, with other minor deflections of a variable nature. In many cases it is apparent that the nerves follow definite paths, which are preformed in the sense

first nerves to grow did so independently of the peripheral conditions, as my own experiments show. The inhibition has affected principally the fibers which grow later, when distances are much greater and conditions much more complex; in other words, it has affected those fibers which grow in the period when differentiation is more dependent upon function, rather than those of the earlier period of self-differentiation.

¹⁹ Cf. Ramon y Cajal, 1908.

that they are marked out by the configuration of other organs. Grooves or spaces between the more solid embryonic organs seem to be paths of predilection. Thus the dorsal nerves, just described, after leaving the medullary cord, run in the small spaces left between adjacent myotomes and the epidermis. Likewise the spinal nerves—at first only the motor constituents—run ventrally in the groove between successive myotomes on the inner side, to reach the extreme ventral part of the musculature. In these cases it does not seem necessary to assume that any special directive factors of a chemotactic nature play a part. However, we are far from being justified in generalizing too freely from the facts just stated, for while the nerves which have been mentioned apparently follow paths of low mechanical resistance, others again, grow where the resistance is probably considerable, as when the first fibers in the central nervous system bore their way through the solid ependyma cells.²⁰

A striking feature of the development of the peripheral nervous system is the fact that the principal nerve paths are laid down very early. In the frog the main branches of the cranial nerves, the sensory spinal nerves from the dorsal cells, and the motor spinal nerves are all formed within two or three days of the closure of the medullary folds, a considerable time before the complete absorption of the yolk. The sensory nerves from the spinal ganglia follow the above named nerves after an interval of a day or two. The point to be emphasized in this connection is that none of the peripheral nerves have very great distances to grow before connecting with their proper end organs.²¹ The *. ophthalmicus*

²⁰ Harrison, '01.

²¹ In criticising a previous statement to this effect Hensen ('08) expressed himself as follows: "Ich glaube mich richtig auszudrücken, wenn ich sage: es ist kümmerlich sich damit helfen zu wollen dass *nur kurze* Wegstrecken (Wie kurz doch wohl?) zu durchwachsen sind. Der Zusammenhang muss *zwangsmässig* gesichert sein." When we consider that, as the present experiments show, nerve fibers have power of independent growth of over a millimeter, that there are obviously conditions in the embryo which may direct this growth for distances of that magnitude, and that scarcely any peripheral nerves have normally much greater distances to grow, then it does not seem to me to be either futile or preposterous to assume that such conditions are sufficient to conduct the developing fiber to its proper end organ.

grows out from the trigeminal ganglion across the optic stalk toward the skin of the front part of the head, spreading out in the region above and in front of the eye and above the nose. The *r. lateralis vagi* connects almost immediately with the rudiment of the lateral line organs and is drawn out as the latter extends towards the tail. The outgrowth of the nerve fibers from the dorsal cells has just been described, and as pointed out, the definitive arrangement can be accounted for by power of growth in a straight line modified by deflection as a result of minor obstacles in the path. The ventral branches of the spinal nerves reach the grooves between successive myotomes and pass ventrally in them to the ventral border of the muscles. As the latter are carried in mass to near the ventral median line the nerves are elongated. These nerves, like the others referred to, have no great distance to grow, and one of the guiding factors, the myosepta, is obvious. The formation of the limb plexuses, comes about by slight deflections from the main path of growth. The nerves to the limbs reach the base of the limb buds in the same manner as the ventral nerves reach the abdominal musculature, *i.e.*, along the grooves on the inner surface of the muscle plates. The nerves are accordingly present in the limbs practically from the time when the latter begin their development, and as the limbs grow the nerves lengthen with them.²² Thus the principal paths are all at first relatively short and subsequently become lengthened by the shifting of parts which takes place during the development and growth of the organism.

It is obvious that the primitive peripheral nerves, which are laid down in early embryonic life, consist of but very few fibers—in the frog often of not more than two or three at first. These first fibers may be called the path-finders; the remaining ones fol-

²² Regarding the period of development at which the nerves reach the limb buds, my own observations ('07 a) differ from those of Braus ('05). In referring to this discrepancy I failed to consider that it might be due to specific differences between *Rana* and *Bufo* on the one hand and *Bombinator* on the other, instead of to errors of observation, as implied in my criticism. Braus has since called my attention to these specific differences, and I am glad to have this opportunity to express my regret at having overlooked this reasonable explanation of the difference in our observations.

low them little by little. Those that develop later, after the growth and shifting of the various parts of the organism has taken place, have much longer distances to grow, but the paths are already laid down by the pioneers and the later ones have only to follow where the others have led.

Plexus formation by outgrowth is admirably illustrated in the nerves arising from the dorsal cells. These fibers begin their growth in a direction perpendicular to the axis of the body, and run intersegmentally approximately parallel to one another, being directed at first from the neural tube towards the epidermis (figs. 1 and 3). Reaching the latter they begin to branch (fig. 6), though not perfectly regularly, owing to slight variations either in the nerve protoplasm itself or in the pathway. In branching, a small deviation from the purely transverse direction of outgrowth takes place, and since the nerves run along the inner surface of the epidermis, and in fact are squeezed in between this layer and the muscle plates, the branches from adjacent segmental nerves must soon come together, and cross one another or form anastomoses. This last stage, which is not figured here from an actual specimen, may be readily observed in parasagittal sections taken just under the skin of *Rana* embryos about 6-7 mm. in length. It has been possible to observe the like of these processes also in the live specimens in lymph; for instance, the formation of branches, the crossing of two fibers growing in different directions, and the fusion or intimate contact (anastomosis) between separate fibers that happen to come together. The diagram on p. 798 (text fig. 1) illustrates the process; the early stages are shown in figs. A and B, and the completion of the plexus in fig. C. This outline of development will account quite satisfactorily for the general features of a cutaneous plexus, *i.e.*, definite areas for each segmental nerve, a considerable overlapping of one segment of distribution upon adjacent ones, and minor irregularities in the mode of branching and anastomosis. A further feature, the oblique course of the nerve trunks in the lower part of the body and the tail, while perhaps in part due to the original direction of outgrowth, is largely brought about by the general shifting of the epidermis over underlying organs, which

takes place during development after the first connections between the nerve fibers and the epidermal cells have been established.

There is nothing in the present work which throws any light upon the process by which the final connection between the nerve fiber and its end organ is established. That it must be a sort of specific reaction between each kind of nerve fiber and the particular structure to be innervated seems clear from the fact that sensory and motor fibers, though running close together in the same bundle, nevertheless form proper peripheral connections, the one with the epidermis and the other with the muscle. That the connection is not long deferred is shown in a large number of instances where the nerves reach their end structures, and function is established very early in development. The foregoing facts suggest that there may be a certain analogy here with the union of egg and sperm cell. The nerve fiber during its growth comes into contact with a cell of the proper kind. Assuming the latter to be in a condition of ripeness, a more intimate contact or perhaps even actual fusion may take place between nerve twig and end cell. A connection of this kind once established would terminate the susceptibility of the cell to further innervation, and nerve fibers growing subsequently in the same path would pass along to other end cells which were mature but not yet innervated. The nerve fiber itself, however, apparently retains its power of growth and ramification, for it usually becomes connected finally with a large number of end cells, as is plainly the case with muscle and ordinary cutaneous endings. It is in the establishment of the definitive connection with end organ rather than in the determination of the direction taken by the main nerve trunks, that influences such as chemotaxis may be expected to operate.

The present experiments suggest, of course, a possible method for further study of this problem. If it could be shown that there is an attraction between growing nerve fibers taken from a certain part of the nervous system and a particular kind of peripheral cell, and between another type of central neuroblast and a different peripheral cell, then we should have direct evidence for the existence of those more subtile factors which seem to

be necessary to account for the definitive establishment of particular nervous connections. The few experiments which I have directed to this end have given negative results, which is not surprising when the crudities of the method are borne in mind, but since it is possible to introduce many refinements into these methods, an ultimate solution of the problem in this way does not seem to be beyond hope of attainment.

The specific arrangement of the fibers within the central nervous system affords a morphogenetic problem of much greater difficulty. Still there is nothing in the conditions in the walls of the neural tube which is inconsistent with the development of the nerve fibers in accordance with the view here represented. The growing fibers are clearly endowed with considerable energy and have the power to make their way through the solid or semi-solid protoplasm of the cells of the neural tube. But we are at present in the dark with regard to the conditions which guide them to specific points.

In pointing out the above factors which seem to be involved in the development of the nervous system, I am aware of the great imperfections in our knowledge of the subject, and of the little progress that has been made beyond the ideas of His and Ramon y Cajal. Nevertheless, although our present conception of the secondary factors which influence the nerve paths may have to be modified in the light of future knowledge, the primary factor, protoplasmic movement, must be regarded as definitely established and it will have to form the basis of any adequate theory of nerve development. The chief claim to progress that the present work has is that it has taken this factor out of the realm of inference and placed it upon the secure foundation of direct observation. With this it has been shown that the first manifestations of activity observable in the differentiating nerve cell are of the same fundamental nature as those found not only in other embryonic cells but also in the protoplasm of the widest variety of organisms. The movement which results in the drawing out of a compact cell into a long filament, the primitive nerve fiber, it is but a specific form of that general type of movement common to all primitive protoplasm. In studying the secondary

factors which influence the laying down of the specific nerve paths of any organism, we are concerned, therefore, primarily with the laws which govern the direction and intensity of protoplasmic movement, and it is the analysis of these phenomena to which students of the ontogenetic and regenerative development of the nervous system must now direct their attention. The present discussion will not have been in vain if it makes clear that the development of the nervous system in the light of the protoplasmic movement concept is no less capable of rational analysis than is development in general.

SUMMARY

Reference is made throughout the following exclusively to the anouran embryo.

Before histological differentiation of the medullary tube begins, its walls do not constitute a syncytium, but are composed of separate cells each with a distinct cell membrane, as freshly teased preparations show.

The peripheral nerve fibers in their earliest stages, as seen in sections of normal embryos, extend from the neural tube or cranial ganglia as finely branched processes of single cells, which in slightly later stages become extended to long fibers; the end of each fiber is a rhizopod-like structure with very fine processes or pseudopodia.

Pieces of undifferentiated embryonic tissue, when isolated under aseptic precautions in clotted lymph, will live for weeks and undergo at least the initial stages of normal histological differentiation: cells from the axial mesoderm give rise to striated muscle fibers; epidermal cells form a cuticular border; typical chromatophores and a mesenchyme-like tissue are formed from pieces containing portions of the neural tube and axial mesoderm; the walls of the neural tube and the primordia of the cranial ganglia give rise to long hyaline filaments closely resembling embryonic nerve fibers.

Tissues grown in lymph function characteristically, as is seen in the movement of cilia and in the contraction of muscle fibers when left in organic continuity with fragments of the neural tube.

One characteristic that the embryonic cells have in common, is the power of movement. They change their form or move from place to place in the clot by virtue of the amoeboid activity of their hyaline ectoplasm. The amount of activity and its result vary according to the tissue, cells from the nervous system and the mesoderm being most active, while those of the endoderm and notochord are most inert.

In the case of cells from the medullary tube and the primordia of the cranial ganglia the activity is so localized and the ductility of the ectoplasm is such, that the movement results in the formation of long fibers, the primitive axones. The free end of each fiber is enlarged and provided with fine processes or pseudopodia. This part continues its progression and the fiber is gradually drawn out.

The rate of progression (lengthening of the fiber) varies considerably, the extremes observed being 15.6μ per hour (100μ in 6 hours 25 minutes) and 56μ per hour (44μ in 47 minutes).

The longest fiber observed, and this was followed throughout its whole period of growth (53 hours), was 1.15 mm. long.

The nerve fibers take origin usually from masses of cells which are so opaque that their mode of connection with the cells cannot be made out, but in a considerable number of cases the fibers were seen to originate in single isolated cells, the longest one of this kind having had a total length of 631μ and having had several long branches.

In many cases anastomoses have been found between fibers. These have been observed to form through secondary fusion, but two threads oncoming together do not necessarily fuse, and anastomoses already formed may be resolved later.

The experiments show that neuroblasts are competent to form primitive nerve fibers within a foreign unorganized medium simply by the amoeboid outgrowth of their protoplasm. By eliminating from the periphery all formed structures which have heretofore been supposed to transform themselves into nerve fibers and leaving only the neuroblasts in the field, it is demonstrated that the latter are the sole elements essential to the formation of nerves. The concepts of both Hensen and Held are rendered untenable.

Taken together with recent histogenetic studies, the experiments show that two elementary phenomena are involved in nerve development: (a) the formation of the primitive nerve fiber through extension of the neuroblastic protoplasm into a filament—protoplasmic movement; (b) the formation of the neurofibrillae within this filament—tissue differentiation.

It is through the former that the specific nerve paths of the body are first laid down. The further analysis of the influences which determine these paths can be made only through the study of the laws which govern protoplasmic movement.

The energy of outgrowth is immanent in the nerve cell, and the initial direction of outgrowth is already determined within the cell before the outgrowth actually begins. The formation of the fiber is therefore an act of self differentiation within Roux's definition.

One of the necessary conditions of outgrowth is in all probability a medium which affords some solid support to the fibers.

The configuration of the various organs of the embryo affords certain paths of predilection, such as small channels or grooves, in which nerve fibers are found to grow. These factors, together with the predetermination of the initial directions of outgrowth within the cell and the motive force of the neuroblastic protoplasm itself, will account for the main features in the topography of the peripheral nervous system.

The first nerves which form are composed of few fibers and have relatively short distances to grow before establishing connection with their end organs. The long paths found in the adult are largely the result of subsequent stretching or interstitial expansion, which takes place as the various parts grow or shift apart. The fibers which develop later follow, in the main, the paths laid down by the pioneers.

The mechanism by which the proper connection between nerve fiber and end organ is brought about is not revealed by the experiments, though a certain analogy with the penetration of the egg by the sperm is suggested.

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EXPLANATION OF PLATES

ABBREVIATIONS WHICH APPLY TO ALL FIGURES

<i>chr</i>	isolated pigment cell;	<i>l</i>	thin layer of cells formed by spreading of transplanted tissue;
<i>cl</i>	embryonic cell exhibiting independent movement in the lymph;	<i>mc</i>	medullary cord;
<i>cut</i>	cuticular border of epidermal cells;	<i>mes</i>	mesenchyme;
<i>ep</i>	epidermis;	<i>ms</i>	main mass of transplanted tissue;
<i>ep.c</i>	epidermal cells in lymph preparation;	<i>my</i>	myotomè, the suffix denoting the serial number of the segment;
<i>ery</i>	red blood corpuscle;	<i>nbl</i>	neuroblast;
<i>fen</i>	opening found in mass of transplanted tissue;	<i>nf</i>	nerve fiber;
<i>fil</i>	hyaline protoplasmic filament stretching between two cells or cell masses;	<i>npl</i>	protoplasmic end of growing nerve;
<i>gn</i>	ganglion;	<i>pl.fr</i>	hyaline protoplasmic fringe, often seen in transplanted epidermis;
		<i>thr</i>	fibrin threads.

PLATE 1

EXPLANATION OF FIGURES

All figures drawn with camera lucida from sections of normal embryos.

1 Part of a frontal section of an embryo of *R. palustris*, 3.6 mm. long. (Series 5b, Row 3, Section 7.) The section is taken through the dorsal half of the medullary cord at the level of the dorsal cells of Rohon-Beard. One of these cells is shown sending out a branched process (*nf*) into the notch between the ninth and tenth myotomes (*my₉* and *my₁₀*). $\times 465$.

2 Part of a frontal section through the head region of an embryo of *R. esculenta*, 3.0 mm. long. (Control Experiment Y, 18 hours, Row 2, Section 1.) The figure shows a portion of the trigeminal ganglion (*gn*) with the fibers of the r. ophthalmicus (*nf*) just beginning to sprout. $\times 465$.

3 Part of a frontal section through an embryo of *R. palustris*, 4.2 mm. long. (Series 6b, Row 4, Section 2.) The section is taken at the same level as that shown in fig. 1. Four dorsal neuroblasts (*nbl*) send out long nerve fibers (*nf*) between two myotomes (*my₂* and *my₃*). $\times 465$.

4 Part of a sagittal section through an embryo of *R. pipiens* (*virescens*), 4 mm. long. (Series 7c, Row 5, Section 14.) The end of one of the dorsal nerves consisting of several fibers is shown. The nerve ending occupies the space between two myotomes (*my₆* and *my₁₀*) and a thick ridge of the epidermis (*ep*). $\times 465$.

5 End of a similar nerve fiber, from another section of same series as fig. 4. Taken between *my₁₃* and *my₁₄*, Row 3, Section 10. $\times 930$.

6 Branching nerve between myotomes 4 and 5 from a section of the same embryo as figs. 4 and 5. The drawing, as indicated by breaks in the fibers, is combined from two successive sections. (Row 5, Sections 12 and 13.) $\times 465$.

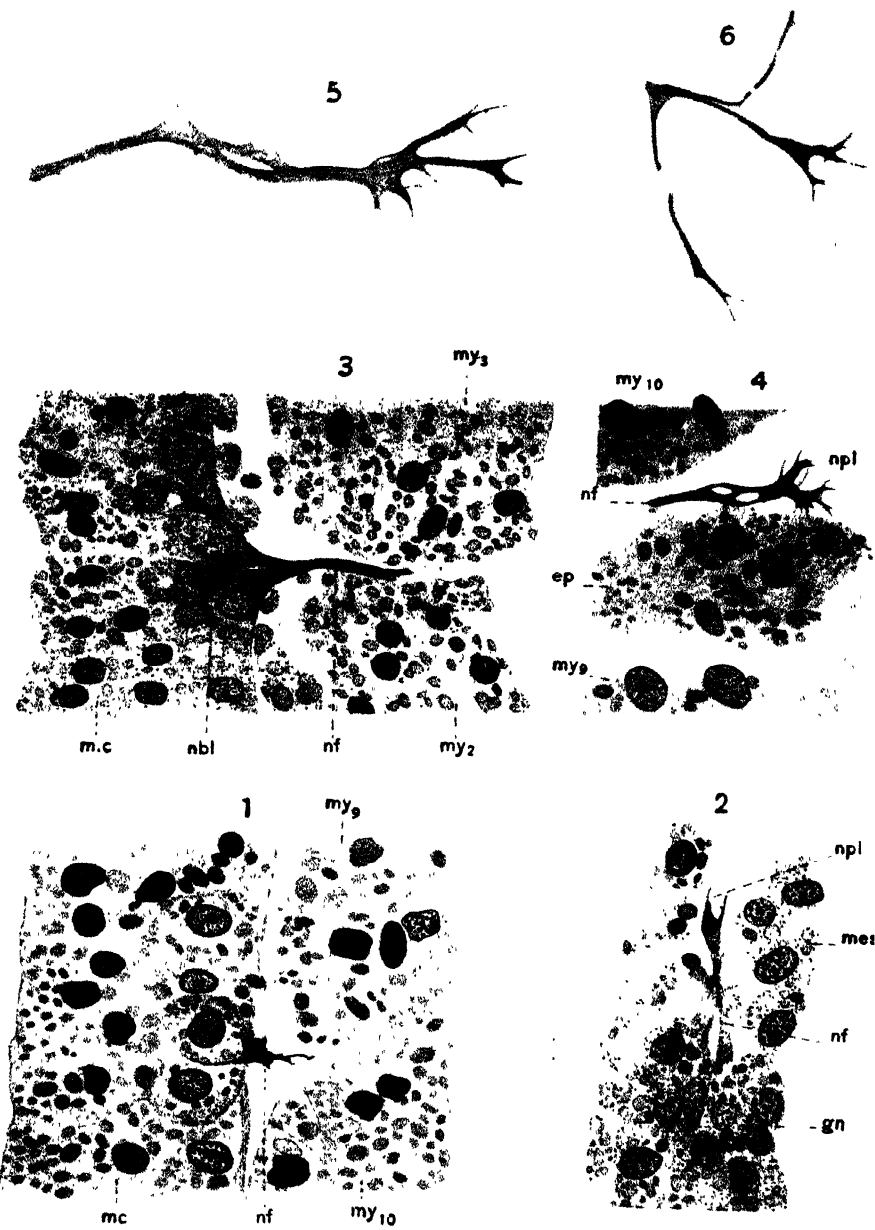


PLATE 2

EXPLANATION OF FIGURES

All figures except figs. 12 and 15 were drawn from camera lucida sketches of living specimens of embryonic tissue isolated in clotted lymph.

7-11 Five views of the same group of nerve fibers made at different times (experiment Is, 137), medullary cord tissue from *R. palustris*, 3.3 mm. long, lymph from *R. pipiens* (the interval between the first and last figure represents 34 hours). $\times 350$.

7 Apparently single fiber (*nf*) growing out from a pointed cell (*ct₁*) which projects from a mass of cells (*ms*) one day after isolation of tissue. April 28, 1908, 12.25 p.m.

8 Same fiber, 2 p.m. Fiber is now clearly double.

9 Same group of fibers. 10.25 p.m. Four distinct fibers (*nf₁-nf₄*) are now visible. The fibrin filaments (*thr*) shown in this figure were present in the earlier stages but were omitted from the original sketches.

10 Same group. April 29, 11 a.m. *nf*, possibly a branch of *nf*.

11 Same group. 10.30 p.m. Continuation of *nf₁* and upper branch of *nf₂*, unfortunately left out of sketch. Note migration of cell (*ct₂*). Identity of other isolated cells in figs. 10 and 11 is uncertain.

12 Three cells from a specimen preserved five days after isolation. Osmic vapor followed by Tellyesniczky's fluid, stained in O. Schultze's haematoxylin. The cells have much branched processes which end indefinitely in the coagulum which pervades whole specimen. Many isolated cells of this kind are in the specimen, which is quite typical. Experiment Is, 157, medullary cord from *R. palustris* embryo; *R. clamitans* lymph. $\times 350$.

13 Row of ectoderm cells from the abdominal region, showing fringe of amoeboid hyaline protoplasm (*pl.fr.*). Experiment Is, 75, two days after isolation. Tissue from *palustris* embryo in *palustris* lymph. $\times 350$.

14 Similar specimen. In this case the ectoderm cells, which are taken from the branchial region, show the cuticular seam (*cut*). The hyaline fringe (*pl.fr.*) belongs to cells lying below the main row. Experiment Is, 87, one day after isolation. Tissue from *palustris* embryo in *palustris* lymph. $\times 350$.

15 Three cells from the medullary cord of frog embryos about 3.3 mm. long, in which the medullary folds had closed and the tail bud was just beginning to appear, prepared from the living specimens; *a* and *b* taken from an embryo of *R. sylvatica*, dissected and examined in 0.2 per cent NaCl; *c* taken from an embryo of *R. palustris*, examined in tap water. The latter cell (*c*) has imbibed water and the cell membrane is very distinct at one side. Nucleus shows as a clear space in each cell. $\times 350$.

16 Whole piece of tissue (medullary cord, with small portions of muscle plates attached) isolated in lymph, two days after preparation. The dark area represents a thick opaque mass of tissue. Thin sheets of cells (*l*) and isolated cells are shown on all sides. *nf*, nerve fibers projecting out into lymph from under the masses of cells. *fil*, threads of hyaline protoplasm bridging spaces between masses of cells. *cd*, band of cells in single file. Experiment Is, 124. Tissue from *pipiens* embryo, lymph undetermined. $\times 32$.

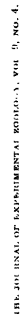


PLATE 2

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10 Same group. April 29, 11 a.m. *nf*₃ possibly a branch of *nf*₁.

11 Same group. 10.30 p.m. Continuation of *nf*₁ and upper branch of *nf*₃, unfortunately left out of sketch. Note migration of cell (*ct*₂). Identity of other isolated cells in figs. 10 and 11 is uncertain.

12 Three cells from a specimen preserved five days after isolation. Osmic vapor followed by Tellyesniczky's fluid, stained in O. Schultze's haematoxylin. The cells have much branched processes which end indefinitely in the coagulum which pervades whole specimen. Many isolated cells of this kind are in the specimen, which is quite typical. Experiment Is, 157, medullary cord from *R. palustris* embryo; *R. clamitans* lymph. $\times 350$.

13 Row of ectoderm cells from the abdominal region, showing fringe of amoeboid hyaline protoplasm (*pl.fr.*). Experiment Is, 75, two days after isolation. Tissue from *palustris* embryo in *palustris* lymph. $\times 350$.

14 Similar specimen. In this case the ectoderm cells, which are taken from the branchial region, show the cuticular seam (*cut*). The hyaline fringe (*pl.fr.*) belongs to cells lying below the main row. Experiment Is, 87, one day after isolation. Tissue from *palustris* embryo in *palustris* lymph. $\times 350$.

15 Three cells from the medullary cord of frog embryos about 3.3 mm. long, in which the medullary folds had closed and the tail bud was just beginning to appear, prepared from the living specimens; *a* and *b* taken from an embryo of *R. sylvatica*, dissected and examined in 0.2 per cent NaCl; *c* taken from an embryo of *R. palustris*, examined in tap water. The latter cell (*c*) has imbibed water and the cell membrane is very distinct at one side. Nucleus shows as a clear space in each cell. $\times 350$.

16 Whole piece of tissue (medullary cord, with small portions of muscle plates attached) isolated in lymph, two days after preparation. The dark area represents a thick opaque mass of tissue. Thin sheets of cells (*l*) and isolated cells are shown on all sides. *nf*, nerve fibers projecting out into lymph from under the masses of cells. *fil*, threads of hyaline protoplasm bridging spaces between masses of cells. *cd*, band of cells in single file. Experiment Is, 124. Tissue from *pipiens* embryo, lymph undetermined. $\times 32$.

PLATE 3

EXPLANATION OF FIGURES

All figures were drawn from camera lucida sketches of the living specimens isolated in clotted lymph.

17 Plexus of nerve fibers growing out from a mass of transplanted medullary cord Experiment Is, 124. Two days after operation. *Pipiens* tissue, lymph undetermined. $\times 350$

18 Bipolar cell with protoplasmic processes. *a*, Free end of process; *b*, process connecting with mass of cells not shown in figure. Length 300μ . Fiber probably formed through movement of cell. Experiment Is, 124, four days after isolation of tissue. *Pipiens* tissue in undetermined lymph. $\times 350$.

19 Bipolar cell and protoplasmic fiber. In this case the fibers were both stretched between two groups of cells and may have been formed by drawing apart. No free ends were visible. Experiment Is, 87, two days after isolation. Tissue from branchial region of embryo. Embryo and lymph *R. palustris*. $\times 350$.

20 Long nerve fiber arising from unipolar cell (*ct*) at edge of group of cells (*ms*). Experiment Is, 124. Tissue from medullary cord, three days after isolation. *Pipiens* tissue, undetermined lymph. $\times 350$.

21 Isolated unipolar nerve cell with long bifurcated nerve filament. Tissue from *R. palustris* in lymph from *R. pipiens*. Experiment Is, 137. Seen at 4 p.m., two days after isolation. $\times 350$.

22 Same cell as in fig. 21, as seen at 8.45 p.m. ($4\frac{1}{4}$ hours later).

23 Two cells from a preparation of medullary cord. Experiment Is, 153, three days after isolation. Tissue from *R. palustris*, lymph from *R. clamitans*. These forms are typical of the isolated cells found in the majority of the preparations. $\times 350$.

24 Two pigment cells from a preparation of medullary cord, including some mesodermic tissue, thirteen days after isolation. Experiment Is, 133. Tissue and lymph *R. pipiens*. $\times 180$.

25 Same cell as *a* of fig. 24, fifteen days after isolation. $\times 180$.

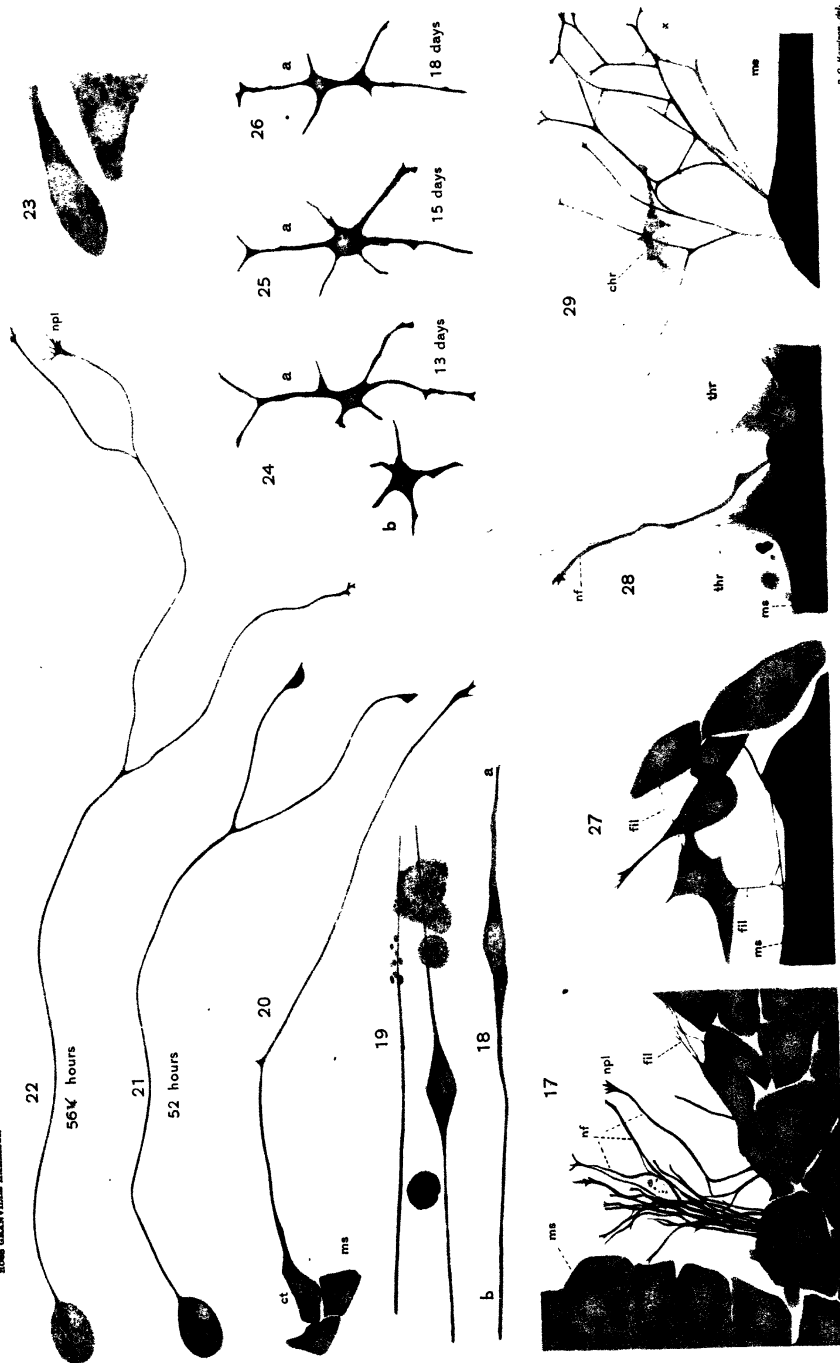
26 Same cell as *a* of fig. 24, eighteen days after isolation. $\times 180$.

27 Group of cells from medullary cord showing protoplasmic processes and connecting threads. Experiment Is, 124. Three days after isolation. Tissue *R. pipiens*, lymph undetermined. $\times 350$.

28 Nerve fiber (*nf*) extending out from mass of cells (*ms*) to show contrast with fibrin thread (*thr*). The fibrin shown is attached to the ectoplasm of several cells upon which it apparently exerts considerable tension. Experiment Is, 69, three days after isolation. The outgrowth of the nerve fiber was observed the previous day. It now shows signs of incipient degeneration. Tissue and lymph from *R. palustris*. $\times 350$.

29 Plexus of nerve fibers arising from a mass of cells taken from medullary cord. The anastomoses were not permanent. The one at *x* was seen to separate, and the day following, nearly all had been resolved. *Chr*, pigment c. ll. Experiment Is, 200, three days after isolation. Tissue and lymph *R. pipiens*. $\times 200$.

OUTGROWTH OF THE NERVE FIBER
FROM GRANVILLE HARRISON



THE STRUCTURE AND FUNCTION OF THE ADULT HEAD KIDNEY OF BDELLOSTOMA STOUTI

GEORGE C. PRICE

Professor of Zoology, Leland Stanford Jr. University

FOUR FIGURES

At the anterior end of the kidneys of the myxinoids, in the pericardial cavity, is a pair of small bodies, which have been known for some years as the head kidneys or pronephroi. They were first observed by A. Retzius ('26) in *Myxine glutinosa*, and were interpreted by him with some hesitation as the functional kidneys.

Johannes Müller ('45), who was the first to give an accurate description of the functional kidneys or mesonephroi, looked upon the kidneys of Retzius as suprarenal bodies. He worked on both *Myxine glutinosa* and *Bdellostoma fosteri*.

Thirty years later Wilhelm Müller ('75), working on *Myxine*, described these organs more fully and more accurately than had been done by either of his predecessors, and came to the conclusion that they represented head kidneys or pronephroi. This interpretation has been generally accepted, and these organs have been homologized with organs of the same name in the larvae of amphibia and bony fishes.

Since the appearance of Wilhelm Müller's article the head kidney of *Myxine* has been made the subject of investigation by Kirkaldy ('94), Semon ('96), Spengel ('97), and Maas ('97); while Weldon ('84) has described it for the genus *Bdellostoma*, his work having been done on *Bdellostoma fosteri*, from the Cape of Good Hope. All these workers, with the exception of Maas, have labored under the disadvantage of having only adults at their disposal. The latter was particularly fortunate in obtaining three young specimens, the smallest only 8.5 cm. in length,

in which the head kidneys were less complicated than in the adult, and he was thus enabled to settle conclusively certain disputed points. His paper contains an excellent review of the literature of the subject.

The present work has been done on *Bdellostoma stouti*. This is the only myxinoid in which the development of the excretory organs is at present known (Price '97, '04). and hence it offers a peculiar advantage for the study of the adult organs, as their somewhat complicated structure may be interpreted in the light of embryology. This, together with the discovery of a probable function of the head kidney, is the excuse for the present paper.

With the exception of a very few species of bony fishes, the myxinoids are the only Craniota described as having a persistent head kidney. As was pointed out by Johannes Müller, and as may readily be seen by a glance at one of his figures, which has been widely copied in text-books of comparative anatomy and embryology, the functional kidney in the myxinoids is very simple, much simpler than in any other of the Craniota; in fact it is more like the kidney of an embryo than of an adult. From this, as well as from the systematic position of the group, one might expect that the head kidney would likewise be simple, and that a study of the excretory organs of the adult would throw light on the question of the homology of the pronephros and mesonephros. But this has not proved to be the case; the head kidney is less simple than the functional kidney, and the primitive relations between them, which is clear in the embryo, is lost in the adult. A study of their development proves conclusively that these two organs are homologous, but this is the only way in which one can be sure of the fact.

In the embryo the excretory organs arise as a series of segmentally arranged tubules, opening into the coelon, and extending in the specimens studied from segments 11-13 to segments 79-82, the exact point both of beginning and of ending varying in different individuals and also on the two sides in the same individual. The excretory duct appears later than the tubules and arises from them. Thus it will be seen that in its origin the entire organ has the characteristics of a pronephros. One pronephric character-

istic, however, is wanting, for at no time is there a glomus in the coelom opposite the nephrostomes.

Later the tubules back of segments 30-33 (this point also varying) lose their connection with the body cavity; or to be more accurate, the small coelomic pocket into which each of these tubules open become cut off from the general body cavity, and forms the distal end of the tubule. In the end of each tubule thus formed, that is, in a cut off portion of the coelom, a glomerulus appears. This portion of the organ has now the structure of a simple mesonephros, a structure which it retains throughout life. However, a few of the more posterior tubules degenerate, and, as will be mentioned below, two or three of the anterior ones become incorporated into the head kidney of the adult.

The tubules in front of the ones just described retain their connection with the coelom, and for a time they also retain their segmental arrangement. But later the gill slits, which are at first far forward, shift their position backwards, and in so doing they crowd before them the anterior end of the kidneys, so that all of the open tubules (17 or 18 in number) and 2 or 3 of those which have been cut off from the coelom and in which glomeruli have been formed, are crowded together into a compact body occupying the space of one or two segments. It is this body which, after undergoing some further changes, forms the head kidney of the adult. It will be seen that this is in a sense a composite structure, since it is formed of two kinds of tubules. The first develop into the main body of the gland, while from the second is formed what may be called the glomerulus of the head kidney. This is the only structure of the kind found in the organ, as neither glomus nor glomeruli are formed in connection with the open tubules.

In the present work adults ranging in length from 22.8 cm. to 56.5 cm. were used, and also two young individuals, the one 7.5 and the other 7.9 cm. in length, in which the intestine still contained an abundance of yolk. While the head kidney in these small specimens has the essential characteristics of the adult they are still in some ways less complicated, and formed a sort of transition between the oldest embryos before studied (Price, '04) and the adult.

More than two dozen head kidneys, in some cases including also the anterior end of the functional kidney, were sectioned and mounted in complete series. While these agree in essential features there is still much variation in detail, so much that an exact description of one would not answer in detail for any other. Living as well as preserved material was studied.

In the adult, as is well known, the head kidneys are situated a little to either side of the dorsal aorta, and project into the dorsal part of the pericardial cavity. They are intimately connected with veins returning blood from the anterior part of the body, the left with the anterior cardinal, or rather with a wide diverticulum given off from this vein just before it reaches the heart; the right with a similar diverticulum given off from a vein which empties into the portal heart. This vein is called by Weldon and by some other authors the anterior portal, but from its position and distribution it looks to be the fellow of the cardinal of the opposite side, although it does not extend nearly so far forward. The relation of the head kidney to the vein is well shown in fig. 2. In *Bdellostoma fosteri*, according to Weldon, the head kidneys are likewise connected with veins returning blood from the anterior part of the body, while in *Myxine* they are connected with the posterior cardinals returning blood from the posterior part of the body.

The size of the head kidney varies with age, although it is relatively small even in large animals. In one specimen 24.7 cm. long the head kidney measured 3 mm. long by 1 mm. broad, while in one 45.6 cm. long it measured 8 mm. by 2 mm. The ratio, however, between the length of the individual and the size of the head kidney is not constant; nor are the two organs of the same size in the same individual, sometimes the right being larger and sometimes the left.

As a rule the organ forms a single compact body, although this is not invariably the case. In one instance, for example, a small bunch of tubules at the anterior end was separated from the main body by an interval of more than a millimeter. Development offers a ready explanation for cases of this kind. In some of the older embryos one or two of the anterior tubules were observed to be entirely separated from the rest and from the duct, and it seems

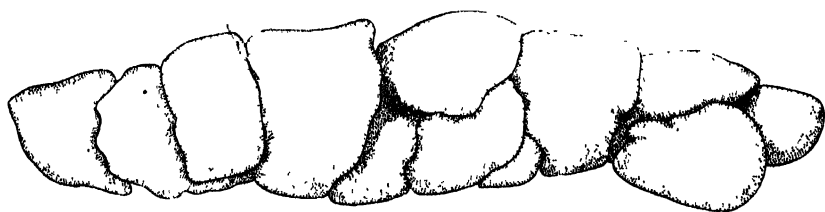


Fig. 1 Head kidney showing the division into lobes. The nephrostomes which lie close together over the surface could not be represented with the magnification used.

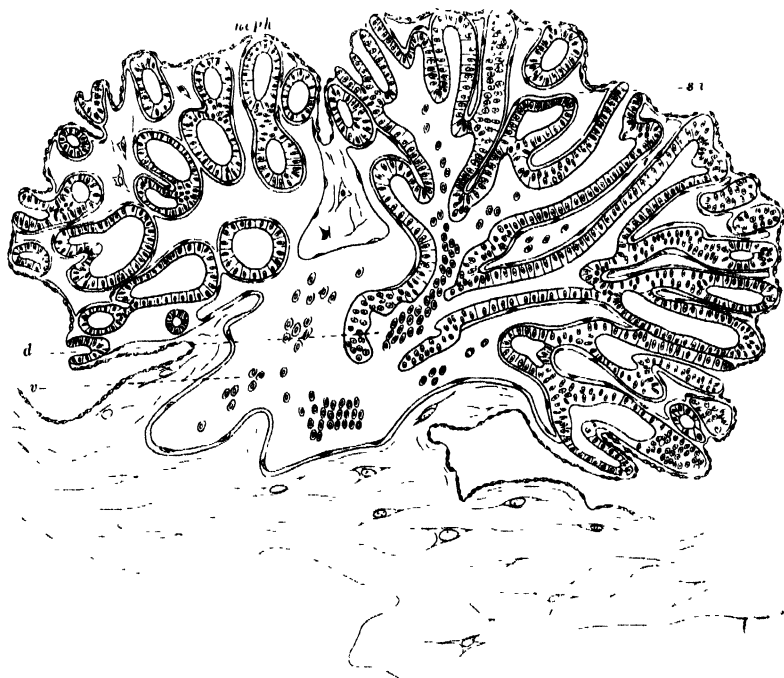


Fig. 2 Transverse section through the head kidney, passing through two lobes; in the one on the right the tubules are cut for the most part longitudinally, while in the one on the left they are cut for the most part transversely. *v*, venous diverticulum with which the head kidney is connected; *d*, central duct containing blood corpuscles and showing connection with both the tubules and the venous diverticulum. A few sections both in front of this and back of it the duct forms a complete ring lying freely in the venous diverticulum. *neph.*, nephrostomes; *sin.*, sinusoids.

certain that the bunch of tubules just mentioned arose from the branching of such an isolated tubule. In another case there was a short but complete break about the middle, so that for five sections there was no trace of any part of the organ. Other examples of the same nature might be given.

In the great majority of cases there is no connection whatever between the head kidney and the functional kidney. There is a short but complete break between the posterior end of the one and the anterior end of the other. In one case no break could be observed, and it was thought the two must be in some way connected, but upon sectioning it was found they simply overlapped, and that there was no actual union. In another case, however, the cavity containing the glomerulus of the head kidney was joined to the duct of the functional kidney by a tubule, and also to the duct of the head kidney. In no instance was the duct of the head kidney connected with the duct of the functional kidney even by a rudiment. But it would not be surprising if such a case should be found, since we know that the two are parts of one and the same duct in the embryo, and that they become separated by the degeneration of a small portion of this duct. When only the adults were known, the presence or absence of even a rudimentary connection between the head kidney and the functional kidney was of considerable interest as throwing light on the probable relation between the two; but in view of the solution of this part of the problem furnished by embryology this ceases to be of any particular significance.

A study of sections shows that the main body of the gland is made up of numerous tubules which open through narrow nephrostomes into the pericardial cavity, and, passing inwards, unite with one another, and finally with the central duct (fig. 2). New tubules are being formed throughout life. These branch out from the old tubules just back of the nephrostome, and while still very short acquire an opening into the pericardial cavity. The new tubules, along with all the rest, grow in length, and may in the course of time give rise by branching to still other tubules. It seems certain that new tubules never grow out from the central duct. If all the tubules in the adult are derived by branching

from the seventeen or eighteen primary tubules of the embryo, one would expect to find about that number of main stems in the adult through which all of the tubules would be connected with the central duct. But this is not the case. Occasionally such a stem is found, but usually instead of a single stem two or three tubules are seen opening into the duct together. This may be brought about in two ways: the main stem (which is never long) may in some way become so shortened that the early secondary tubules appear to open directly into the duct; then again, on account of the crowding together of the tubules in the embryo two primary tubules may come to open into the duct very close together. For these reasons it is difficult in a series of sections to determine what must have been the number of primary tubules. And yet with care this may be done with considerable accuracy. In one case the duct was reconstructed on millimeter paper, and it was found that tubules connected with it at twenty places. This is about the number of primary tubules of both kinds found in the head kidney in the embryo.

When the entire organ is viewed with a lens it presents a more or less distinctly lobed appearance (fig. 1), as has been observed by previous workers in other members of the group. Judging from what may be seen of the lobes and of the course of the tubules in a stained and cleared preparation, it seems probable that each lobe is made up of a single primary tubule, together with all of its branches. The number of distinct lobes, however, is usually not so great as one would expect if this were the case.

The two chief causes for the increase in the size of the head kidney with age are the growth of the tubules in length and their increase in number; the diameter changes little if any. The following figures will give an idea of the increase in length; in an individual 7.5 cm. inches long, in which only part of the tubules showed any indications of branching, the average length of a few of the primary tubules was 0.165 mm.; in an individual 27.9 cm. long the average length of a few of the longest tubules was 0.292 mm.; in one 35.5 cm. long it was 0.464 mm.; and in one 45.7 cm. long it was 0.90 mm.

The tubules are so numerous and so closely packed together that it was entirely out of the question to try to count them after sectioning. While an idea of the number in a small individual could be gained by first staining the entire organ and then counting the nephrostomes under a strong lens, this method could not be used with large individuals, in which the number ran into the thousands. But by first staining the entire organ, and then teasing into small pieces in glycerin, and counting under the compound microscope, a rough idea of the number of tubules in several individuals of different sizes was obtained. In this way 265 tubules were counted in a head kidney from an individual 29.2 cm. long, 791 in one from an individual 34.2 cm. long, 6084 in one from an individual 49.5 cm. long in which the head kidney was unusually large, and 2300 in one from an individual 56.5 cm. long. In all cases the actual number must have been greater than the number counted, for it was not possible to get all the pieces so small that every tubule could be seen. In the case where 265 tubules were counted at least 300 nephrostomes were seen before teasing. It will be noticed that the largest and presumably the oldest individual did not have half so many tubules as the one next smaller. Perhaps the first explanation that would suggest itself for this would be that it was due to degeneration and the consequent disappearance of tubules. But this is not likely the case, for so far as possible in counting, every tubule was observed, and no indication of degeneration was noticed. Further, this is not so surprising when we take into consideration the great amount of variation in all parts of the organ.

The average diameter of a number of tubules from three individuals of different sizes was 0.055 mm., and the average diameter in the largest individual was no greater than in the one under three inches long. As a rule there is but slight variation in the size of the tubules in an individual; but in some instances, especially in larger individuals, a portion of a tubule may be so enlarged as to form a vesicle as much as four times the diameter of an ordinary tubule. These vesicles were absent in more than half the head kidneys examined. When present they varied in number from two to seven. As has been observed in other members of

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